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Sodium Intake of the American Male: Implications on the Etiology of Essential Hypertension

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THE AUTHOR has obtained evidence based on estimated dietary intake of sodium as well as urinary excretion of this element which indicates that the level of sodium intake is the primary etiologic factor in the development of essential hypertension in humans.[1–5] Because of this thesis concerning the cause and effect relationship between sodium ingestion and human hypertension, it has become important to establish the range of sodium eaten by a group of ambulatory, asymptomatic adult Americans as a base-line for comparison with other societies in which essential hypertension is present to a significantly greater or lesser degree than in our own. The present paper is a report on the intake of sodium, based on the urinary excretion of sodium, among 71 adult males. A summary of the data on a few of these subjects has been presented earlier in a paper dealing with the levels of sodium intake in people with and without hypertension.[4]

RATIONALE, PROCEDURE, AND METHODS

In this report, the urinary excretion of sodium has been assumed to be virtually equivalent to sodium intake. During a 10-year period of prolonged metabolic studies on ambulatory, asymptomatic individuals with good renal function, with and without hypertension, the author has found the urinary excretion of sodium to be a highly reliable index of sodium intake. This use of urinary excretion is subject to error under the following circumstances: (1) factors affecting renal function primarily or secondarily, e.g., (a) kidney disease with abnormal wasting or retention of sodium, (b) cardiac failure, cirrhosis, hypoproteinemia, (c) hormonal effects with sodium retention, e.g., premenstrual edema, steroid administration, etc., (2) extra-renal losses, in which the two commonest are through the gastrointestinal tract and the skin; obviously such conditions as (a) ptyalism, (b) gastroenterocolitis, (c) severe sweating, (d) an exuding dermatitis or a large burn with weeping, could lead to significant sodium loss. None of these was present in the subjects in the current investigation. Over a wide range of sodium intakes (from 2 to 180 meq/d) the author, as well as many other investigators (e.g.,[6,7]) has found the normal stool excretion of sodium to be about 3 meq/d so that this route of loss may be disregarded except in individuals who have been on prolonged and drastic sodium restriction. Therefore, in otherwise healthy people the chief source of unaccounted sodium loss will be through sweat. Among the 71 subjects whose urines were analyzed for sodium, 55 were in sedentary occupations (scientists, technicians, administrators) and 16 were in occupations requiring light manual work (orderlies, kitchen helpers, clerks, etc.). Studies on 68 people were done between the months of November and April during the winters of 1954–55 and 1955–56, in the course of which the weather varied from cool to cold: three collections were made in June, 1956 during a period of warm weather. It is unlikely then, that either

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TABLE I

Urine Sodium—Males Only

Case no.	Age	Weight lb	Work*	H.T.†	Single collection meqNa/24 hr	Multiple collections		
						days	meqNa/24 hr	Av
1	55	172	S	0	95	13	120, 111, 144, 164, 123, 110, 134, 122, 12, 4, 98, 114, 140, 133	126
2	43	183	LM	0	96	7	140, 143, 168, 216, 108, 107, 225	158
3	40	159	S	0	98	6	144, 178, 174, 164, 188, 192	173
4	39	159	S	0	115	7	208, 152, 183, 178, 188, 120, 71	157
5	27	144	S	0	116	7	108, 128, 215, 149, 194, 342, 161	185
6	31	178	S	0	117	—	—	—
7	54	148	S	0	117	—	—	—
8	52	155	S	+	118	—	—	—
9	33	No weight, slender	S	+	119	—	—	—
10	39	142	S	0	122	—	—	—
11	35	184	S	0	123	—	—	—
12	35	151	S	0	124	8	159, 162, 115, 111, 205, 86, 125, 111	134
13	62	136	LM	0	124	—	—	—
14	29	187	S	0	124	7	120, 104, 105, 164, 141, 179, 107	131
15	37	145	S	0	134	—	—	—
16	36	162	S	0	136	7	99, 148, 149, 134, 107, 111, 126	125
17	35	137	LM	0	137	—	—	—
18	37	142	LM	0	140	—	—	—
19	56	163	LM	+	141	7	173, 245, 157, 148, 177, 196, 159	179
20	28	119	S	0	142 242 281	7	250, 161, 153, 112, 132, 132, 154	156
21	57	157	S	0	145	—	—	—
22	61	223	S	+	145	—	—	—
23	49	152	S	0	149	7	168, 139, 165, 111, 143, 126, 125	140
24	62	154	S	+	152	—	—	—
25	31	170	S	0	154	7	147, 149, 203, 173, 298, 232, 165	195
26	40	152	S	0	159	—	—	—
27	26	167	S	0	163 275	—	—	—
28	49	153	LM	0	164	—	—	—
29	32	147	S	0	169	—	—	—
30	36	198	LM	+	171	7	224, 144, 192, 156, 238, 292, 169	202
31	26	167	S	0	172	—	—	—
32	40	172	S	0	173 147	38	134, 136, 206, 238, 208,‡ —, —, 130, —, —, —, 151, 126, 117, 130, 193, 281, 184, —, 145, 130, 197, 170, 140, 235, 157, 192, 223, 149, 192, 223, 324, 180, 201, 182, 215, 197, 219, 216, 197, 119, 102, 125, 168	180
33	45	166	S	0	178	—	—	—
34	45	179	LM	0	180	—	—	—

physical activity or ambient temperature led to significant unrecorded loss of sodium through excessive sweating in the overwhelming majority of subjects studied here.

Only males were selected for this program in view of the greater ease of collecting urine samples as well as the lack of need to allow for premenstrual sodium retention. It is possible that this elimination of females may have skewed the data slightly in favor of somewhat higher intakes of NaCl, since the author[5] as well as Ashe and Mosenthal[8] have found that among females there are more low-salt intake people while among males there are more high-salt intake individuals. Because this study was carried out as part of the author's investigations on the causal relationship between sodium ingestion and hypertension, 22 of these men had asymptomatic essential hypertension of varying levels. None of these hypertensive subjects however, had poor renal function as indicated by nocturia, fixed specific gravity or nitrogen retention, although some of them had mild albuminuria. In the first study, 70 men collected 24-hour urines (two subjects collected specimens for two 24-hour periods and another for three separate days). Subsequently, 27 of these men, as well as another man who had not participated in the first study, volunteered to collect 24-hour specimens for a week or

TABLE I—*continued*

Urine Sodium—Males Only

Case no.	Age	Weight *lb*	Work*	H.T.–	Single collection *me Na/24 hr*	Multiple collections		
						days	*me Na/24 hr*	*Av*
35	63	170	S	+	183	—	—	—
36	32	226	LM	+	183	—	—	—
37	48	226	S	+	185	9	241, 234, 177, 254, 210, 140, 179, 174, 163	197
38	41	189	S	+	187	—	—	—
39	33	159	S	+	187	—	—	—
40	33	170	LM	0	189	7	194, 206, 206, 169, 233, 240, 220	210
41	37	166	S	0	189	—	—	—
42	42	194	S	+	191	7	230, 296, 260, 201, 238, 163, 203	227
43	42	197	S	+	193	—	—	—
44	33	169	S	0	195	7	103, 172, 185, 184, 134, 223, 195	171
45	45	212	S	0	197	7	283, 213, 176, 145, 160, 148, 162	184
46	30	178	S	0	200	—	—	—
47	47	173	S	0	202	—	—	—
48	33	138	S	0	203	28	174, 168, 170, 147, 141, 136, 220, 222, 218, 155, 145, 232, 245, 219, 186, 221, 161, 176, 128, 155, 187, 174, 169, 218, 184, 157, 150, 198	181
49	43	198	S	0	206	7	63, 103, 96, 115, 117, 122, 195	116
50	30	169	S	0	208	—	—	—
51	45	170	S	0	210	—	—	—
52	40	163	LM	+	217	—	—	—
53	50	183	LM	+	217	—	—	—
54	53	189	LM	+	222	7	179, 222, 152, 154, 165, 214, 259	192
55	49	148	LM	0	225	—	—	—
					226			
56	53	200	S	0	236	—	—	—
57	27	156	S	0	238	—	—	—
58	62	178	LM	0	240	—	—	—
59	31	209	S	0	241	—	—	—
60	24	257	S	+	246	—	—	—
61	47	230	S	+	253	—	—	—
62	39	189	S	0	262	—	—	—
63	69	187	S	+	270	7	169, 173, 175, 203, 406, 182, 108	202
64	33	152	S	+	272	7	170, 195, 249, 309, 259, 213, 259	236
65	40	195	S	0	279	—	—	—
66	25	143	LM	0	289	7	225, 271, 294, 171, 266, 151, 276	236
67	37	239	S	0	296	8	194, 153, 134, 126, 162, 229, 307, 224	191
68	31	142	S	0	307	—	—	—
69	45	177	S	+	330	7	233, 285, 253, 219, 276, 274, 166	244
70	38	201	LM	0	364	—	—	—
71	45	203	S	+	No sample	6	105, 199, 144, 173, 119, 219	160

* S—Sedentary; LM—Light manual. † HT—Hypertension. ‡ Collection not obtained on this day.

longer. Therefore, in these 28 adult males, urine collections were made for from 6 to 38 days (median 7 days; average 9.1 days) and with few exceptions the collections were made on consecutive days. Each man had detailed verbal and written directions on the procedure to be followed. If a sample was lost for any reason, the subject was instructed to discard the entire collection and resume on a full 24 hour basis. Similarly, he was told not to deviate from his usual behavior and activities, and the test was to be stopped only in the event of illness. Urine was voided directly into chemically clean capped bottles specially prepared in the author's laboratory. Most of the men in this series were unaware of the problem under study but included here are the data from five colleagues in the Brookhaven Medical Department who knew of the general interest of the author: all five denied any conscious change in their use of salt or salty foods during the period of these collections. Of the 67 men classified according to salt (NaCl) intake by the author's technic[2] there were 11, 29, and 27 distributed among the low, average and high salt-intake groups, respectively. This distribution corresponds closely with that of the series of 1,124 unselected adult males from whom the 67 were derived.[5]

Sodium concentration in the urine was

measured on a Baird Model DB2 flame photometer using an internal lithium standard. From the measured 24-hour urine volume, and the concentration of sodium therein, the 24-hour urine sodium output was calculated.

RESULTS

The primary data on individual patients are shown in Table I.

In Table II the data for the single and multiple collection groups have been summarized.

TABLE II

Urinary Excretion of Sodium, meq/Day

	Mean	Median	Range
70 Subjects; single collections (74* samples)	186	183	95–364
28 subjects; multiple collections (256 samples)	178	180	63–406

24-hour excretion of sodium in urine (meq/d) in 70 ambulatory male subjects in whom single 24-urine collections were obtained (* in two subjects two 24-hour specimens, and in one subject three daily specimens were collected) compared with excretion values for 28 subjects in whom 24-hour samples were obtained for from 6 to 38 days. (See Table I for primary data.)

The range for the two groups was similar, namely from about 60 to 400 meq Na/day (approximately 4 to 24 g of NaCl). However, the average and median levels of daily urinary sodium excretion for both groups were bunched closely about 180 meq/day—equivalent to about 10 g of NaCl.

These findings suggest that, relative to urinary excretion levels, one group fairly mirrors the other. However, from Table I it is apparent that single 24-hour urine collections often could have been misleading as to the more usual level of sodium ingested, which is shown by variations from day to day of from 2 to nearly 4-fold in some subjects on whom multiple collections were made. Nonetheless in the series with 28 members, among those men with low and high average daily excretions of sodium, it was common to find fairly constant low and high daily levels, respectively. By contrast, subjects whose average excretion was about the group mean tended to vary widely from day to day from low to high levels.

DISCUSSION

Among standard texts the average intake of sodium in adults is said to be about 5–15 g of NaCl per day but primary references are notable by their absence.[9–11] Studies in which various kinds of control[12] are imposed during the collection of samples clearly are not applicable. Except for an occasional report of an individual subject who was allowed an unrestricted salt intake prior to the onset of an experiment, the only pertinent study which the author could find was that of Ashe and Mosenthal.[8] These authors measured the 24-hour urinary excretion of chloride in 1,000 ambulatory adults of New York City, who had no disease other than hypertension. Although for the most part only a single 24-hour collection was analyzed on each person, an unstated number had several such collections during the more than 15 years covered by the study. No primary data were given in the paper, but the following ranges of urinary excretion (calculated as NaCl) were found: (a) 4 g or less—50 subjects; (b) 4 to 8 g—416 subjects; (c) more than 8 g—534 subjects. In contrast to the present author,[4] they found no differences in the amount of salt eaten by the 437 individuals with, and the 563 without, hypertension. They concluded however, that among females there were more "low-salt eaters" and among males more "high-salt eaters," a conclusion with which the author is in agreement.[8]

The urinary excretion data reported here are in line with the commonly quoted estimates of daily sodium chloride intake of adult Americans as ranging from about 5 to 15 g. Although stool and skin losses were not measured in these subjects, as noted earlier it seems unlikely that under the conditions of this study the results would have been changed materially. Direct comparison between these data and those reported by Ashe and Mosenthal is made difficult by the absence of primary data in their paper, but it seems worth while to compare the two series briefly. The 24-hour urine sodium chloride excretions may be divided into three groups according to the schedule used by Ashe and Mosenthal. By such a classification it is possible to say that at one time or another in the present study, 2, 36 and 62 per cent of the

71 subjects excreted sodium in amounts which could place them in groups (a), (b), or (c), respectively. This distribution does not differ significantly ($P > 0.1$) from that of the 1,000 subjects reported by Ashe and Mosenthal. It is of interest however, that among the 330 separate 24-hour collections included in the current study, only one specimen had a level of sodium below the equivalent of 4 g NaCl/d. And, while 36 per cent of the subjects had sodium excretions at one time or another equivalent to 4–8 g NaCl/day, the 77 samples in this range comprised only 23 per cent of the total. Thus it may be unwise to assume that the two series were similar. As noted earlier, this disparity might be accounted for in these 71 subjects by the exclusion of females who have somewhat lower salt intakes than males. Another possibility deserving of consideration is that people are eating more salt (NaCl) today.

The excretion of amounts of sodium in the urine in these subjects indicates a gross positive metabolic balance for the ion, the metabolic need for which is not apparent since the daily requirements can ordinarily be satisfied by 1–2 g of NaCl,[13] or even less. It has been inferred that ingestion of salt at levels found in our society is beneficial by virtue of its effect on adrenal cortical hormone secretion, thereby providing a "stimulating" action which makes the organism more responsive to the increased demands of our complicated society.[14] On the basis of nearly 10 years' experience with drastic (2–6 meq/day) prolonged sodium restriction in the study and treatment of individuals with hypertension as well as in the study of the effects of drastic salt restriction on normotensive control subjects, the author has found no evidence to suggest the validity of such an inference.[6,15–18] The regulation of the immediate response to heightened demands upon the organism is largely controlled by the adrenal medullary hormones, epinephrine and norepinephrine.[19] The author has reported the results of studying the pressor response of nine subjects, eight with hypertension, to nor-epinephrine before and after sodium restriction.[20] Only one of these nine individuals, a 45-year-old female with essential hypertension, showed a clear-cut decrease in the vaso-pressor response to nor-epinephrine after salt limitation. Since the capacity for constricting blood vessels and raising the blood pressure is a very basic one for survival, the lack of change following sodium restriction in eight of the nine subjects suggests no lessened capacity for response on the part of these subjects. Furthermore, among the men studied for from 6 to 38 days in the current report there were individuals who, by dietary history and pattern of urine excretion, routinely ate only half as much salt as some of their contemporaries: there was no evidence of mental, physical, or psychologic superiority of those on the higher salt intake. The author has had under continuous metabolic observation a 69-year-old widow (#5504) who was admitted in September of 1953 to the Brookhaven Laboratory Research Hospital for study and treatment of her essential hypertension of 12 years' duration. It was established that this woman had normal renal and cardiac function, judged by the usual clinical and laboratory studies. After six weeks of control observations during which time she received 180 meq Na/day, her daily sodium intake was sharply reduced to 4 to 6 meq/d on October 26, 1953. After almost four years of such limitation, this woman remains the same active, ambulatory, intelligent (and somewhat aggressive) female that she was prior to sodium reduction, although her blood pressure has been normal for several years. Her daily activities are numerous and ordinarily include two walks, each of one to three miles in length, on the laboratory grounds. From some studies in preparation for publication, it has been found on a number of individuals that as compared with the observations during control periods when the daily sodium intake averaged 180 meq/d (10 g of NaCl), prolonged and drastic (6 meq/d) sodium restriction was unassociated with mental effects discernible by a skilled psychiatric analyst upon detailed interview. Psychologic tests performed by a trained professional psychologist showed no objective changes; electroencephalograms were unaffected by this degree of sodium limitation.

The level of sodium intake found among these Americans may be compared with that of

other societies in which hypertension is more or less frequent. As noted earlier[2] a virtual absence of hypertension has been found among different primitive ethnic groups who also differ widely in environment: e.g., the Greenland Eskimos,[21] Australian aboriginal,[22] mountainous Chinese tribes,[23] and the Cuna Indians of Panama.[24] It was of interest to find that among all of the truly primitive groups for which data were available, a common factor was a low intake of sodium, estimated from listed foods or actual analyses to contain about 1 to 2 g (2.5 to 5 g as NaCl) a day and sometimes less. By contrast, there is considerable evidence that the West Indian Negroes have a much higher incidence of hypertension than either whites or Panamanian Indians.[25,26,27] In discussing this problem in 1953 with Prof. G. P. Murdock of the Yale University Department of Anthropology, I was informed that an associate in his department had recently returned from a field trip to Jamaica, British West Indies, and had spontaneously reported that the Negroes of that area ingested large amounts of salt, chiefly through the consumption of salted pork and fish. Indeed this anthropologist reported that salt imagery played a prominent role in the songs, stories, and jokes of these people. Thus, although precise estimates of salt intake are presently lacking in these people (the author is currently organizing an expedition to determine the precise range of salt intake in these people), the evidence suggests that a high-salt intake is present, and in all probability, considering the restricted diet of these people, such an intake can be assumed to begin in early childhood at a time when, at least in animals, the organism is more sensitive to the hypertensive effects of a high sodium intake.

SUMMARY

As an index of sodium intake, complete 24-hour urine collections were made on 71 ambulatory, working, male adults for periods ranging from 1 to 38 days. The mean and median 24-hour sodium excretions were about 180 meq/day—equal to approximately 10 g of sodium chloride. The minimal and maximal daily excretions of sodium were 63 and 406 meq which are equivalent to about 4 and 24 g of sodium chloride, respectively.

Such levels of sodium excretion indicate intakes of comparable magnitude, since these healthy subjects were presumably in sodium equilibrium. There is increasing evidence that high intakes of sodium are harmful: the West Indian Negroes who probably have high intakes of sodium from early childhood develop hypertension much more frequently than do the white people. Furthermore hypertension is virtually absent among races of people known to have a low-sodium intake. All of this evidence is in agreement with the author's thesis that sodium is one major etiologic factor in the development of essential hypertension in humans.

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Comparison of Temperature Responses to Intravenous Infusions of Dextrose and Fat Emulsions

By HAROLD L. UPJOHN, CAPT., M.C. AND STANLEY M. LEVENSON, M.D.

A FEBRILE response to intravenous fat emulsion is the most frequent untoward side effect following short-term infusions. Though this side effect is probably not now common or severe enough to limit the clinical usefulness of intravenous fat emulsion, an understanding of its mechanism is important. One hypothesis offered is that the fever is a result of rapid metabolism of the infused fat with the resultant production of large amounts of heat, rather than being the result of some specific property of the fat in the emulsion. This hypothesis suggests that any infusion containing the same caloric equivalent as a standard infusion of fat, administered and metabolized at the same rate (or faster) would result in a similar number of febrile responses. The purpose of this study was to compare the temperature responses of a group of healthy subjects to isocaloric intravenous infusions of fat emulsion and dextrose.

Intravenous fat emulsion is commonly administered at the rate of 900 calories in four-hour intervals. If a normal adult receives intravenous dextrose at the maximum rate at which there is little urinary spillage, his rate of caloric intake will approach that afforded by a standard fat emulsion infusion. At an infusion rate of 0.8 g of dextrose per kg body weight per hour, one may expect up to 5 per cent urinary loss. Therefore, we administered 250 g of dextrose at this rate to insure a total retention of about 225 g (900 cal). A dextrose infusion supplying calories at approximately the same rate as a standard fat infusion was thereby achieved. In view of the known diurnal variation in temperature and the differences in temperature among healthy subjects, control days on which temperatures were taken but no infusions given were included in the study.

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METHODS AND MATERIALS

Subjects

The subjects for the study were volunteer convalescent patients selected from the surgical wards of Walter Reed Army Hospital. Their injuries were limited to those of an orthopedic or neurosurgical nature; all were ambulatory, nonfebrile, and otherwise in good health. One had suffered from asthma as a child; another was allergic to penicillin. The majority of the subjects were in their twenties or early thirties. All subjects were measured for height at the beginning of the study and were weighed at the beginning and end of each week.

Location

The study was carried out on an enclosed porch of one ward at Walter Reed Army Hospital. No other patients were bedded on this porch. The average daily ward temperature was 77° F, and the range of the daily average temperature was 75° to 79° F; the highest hourly temperature was 81° F and the lowest 74° F.

Daily Program

Subjects reported to the ward at 8 A. M. and infusions were started soon afterwards so as to be completed just prior to lunchtime. The sub-

jects were permitted coffee during the morning, and lunch was sent in from the mess hall. In the afternoon, subjects were permitted normal activity including appointments for physical therapy, the changing of casts and so forth; the only condition being that they be available hourly for temperature readings. There was no attempt to control the quantity of food that the subjects ate either at or between meals. The study began on March 4, 1957 with a control day. On the following four days, dextrose infusions were given. The eighth day was also a control day and was followed by three days during which fat emulsion infusions were administered.

Infusions

All infusions were administered in the morning and usually lasted about four hours. The exact duration of each infusion was recorded.

(A) *Dextrose:* One 50 ml ampul of 50 per cent dextrose was mixed aseptically with a 500 ml bottle of 20 per cent dextrose to give 550 ml of a 22.7 per cent dextrose solution. On the days of dextrose infusion, each subject received in succession two bottles of a 22.7 per cent dextrose solution, a total of 1,100 ml. This amount contains 250 g of dextrose, hence, is equivalent to 1,000 calories. The 50 ml ampul of 50 per cent dextrose was Army stock issue and was prepared by Philadelphia Ampoule Laboratories. The 20 per cent dextrose was Baxter Travenol®, lot B94297, supplied by Baxter Laboratories, Morton Grove, Illinois, and Cleveland, Mississippi. The dextrose infusions were administered by means of Plexitron Intravenous Injection Sets, disposable type, made by Baxter Laboratories. This set delivers dextrose solution in drops of a size such that 15 drops make 1 ml.

The rate of dextrose infusions was set by the weight of the subject according to the formula of 0.8 g of dextrose per kg body weight per hour. The delivery rates were checked every half to three-quarters of an hour.

(B). *Fat:* The intravenous fat emulsion preparation, lipomul I.V., consisted of 1.2 per cent soybean phosphatide, 0.3 per cent pluronic F68, 15 per cent w/v vegetable oil and 4 per cent w/v dextrose, in 600 ml bottles. This preparation was provided by The Upjohn Company, Kalamazoo, Mich., under their lot number 11,612-30. Each bottle contained the equivalent of 810 cal as fat and 96 cal as dextrose, thus 906 cal in all. The caloric contribution from phosphatide in the emulsion has been disregarded. The lipomul was administered by a Fenwal HB 122 Recipient Set with PRJ 706 bottle vent. This type of set delivered the emulsion at 24 drops per ml.

A fat emulsion infusion was always preceded by a small infusion (10 to 50 ml) of 5 per cent dextrose (Army stock issue, prepared by Cutter Laboratories, Berkeley, California, and Chattanooga, Tennessee) given at a very slow rate. The fat emulsion infusion set was then inserted into the rubber adapter on the dextrose set. A slow rate of delivery of about 10 drops per min was maintained for the first 20 min of the fat emulsion infusions. It was then increased to between 60 and 70 drops per min. This resulted in a delivery time of about four hours for the entire 600 ml bottle. The rate of delivery was regulated by Hoffman clamps and was checked every half hour.

Temperatures, Pulses and Blood Pressures

Rectal temperatures and pulses were taken as near to hourly intervals as was possible. The schedule resulted in an average of 11 readings for the 12-hour period from 8 a.m. to 8 p.m. Blood pressure readings were made whenever convenient; usually three to five determinations a day.

Urine Determinations

Every urine sample voided by each subject during the 12-hour period was collected. The amount of the sample was recorded and simple tests for sugar and acetone bodies were made with Clinitest® and Acetest® tablets.

Blood Determinations

Blood samples were taken by venipuncture on the first control day and on the first and last days of fat infusions. White blood counts, differential counts, and hemoglobin and hematocrit determinations were made.

Calculations and Charting

For each infusion we wanted to determine the total number of available calories which the subject retained and the rate at which these were delivered. For dextrose, all urinary losses for the 12-hour period were subtracted from the total amount delivered on the basis of 4 cal/g of dextrose. The caloric content of the fat emulsion infusions was computed by adding the contribution of the fat, at 9 cal/g, to that of the dextrose, at 4 cal/g.

The temperature data were analyzed in several ways. A temperature profile was drawn for each subject on each day. In this way the control, dextrose, and fat reactions could be easily compared, especially in regard to the time of the highest temperatures and the extent of temperature rise. The frequency of febrile responses was determined by the criterion of at least a two degree rise above the control temperature on the day of the infusion.

RESULTS AND DISCUSSION

There were two control days, with 12 subjects participating on the first and nine on the second; an additional control day was obtained with the tenth patient on a day when he was not given a dextrose infusion, so that there were a total of 22 control records. Forty-six daily infusions of 22.7 per cent dextrose were given in a four-day period to 12 healthy men. The following week, nine of these subjects each received three daily infusions of a 15 per cent fat emulsion. The dextrose and fat emulsion infusions were approximately equal in caloric content and calorie rate of infusion. There were no febrile responses. The temperature patterns of the control days, dextrose days, and fat days were substantially the same.

The average total number of calories given in the dextrose infusions was 980 as opposed to 906 for the fat emulsion infusions, but since the dextrose infusions lasted on the average a bit longer than the fat, 4.2 hours as compared to 3.9 hours, there is an excellent correspondence between the over-all average rates of theoretic caloric infusions of 234 cal/hr for dextrose and 232 cal/hr for fat emulsion.

Parenthetically, we may note that the delivery rates demonstrate the advantage of fat emulsions over dextrose for high caloric infusions. To get an isocaloric rate of intake, we had to give an average of 262 ml/hr of dextrose solution but administered only 154 ml/hr of fat emulsion. In addition, the dextrose had to be quite concentrated, 22.7 per cent, and thus had the liability of being locally irritating. There was some spillage of dextrose into the urine, but in all cases it was less than a 5 per cent urinary loss. At least 225 g of dextrose was retained during each intravenous dextrose infusion. Six fat emulsion infusions in three patients gave rise to urinary acetone bodies. After five of these infusions the urine showed trace amounts and after one a strongly positive reaction for acetone.

TABLE 1

	Average temperature range °F	Average rise	Mean temperature
Control days	98.2– 99.8	1.6	99.0
Dextrose infusion days	98.4–100.0	1.6	99.1
Fat emulsion infusion days	98.4–100.0	1 6	99 1

By all the indices that were assembled to assess the temperature rises, we conclude that no febrile responses occurred and that there were no differences in temperature on the control, dextrose and fat emulsion days. As may be seen in Table I, the average temperature ranges, temperature rises, and average mean temperatures are almost identical in all three circumstances. The profiles on temperature charts point to the same conclusion. In almost all cases, the control, dextrose, and fat emulsion profiles are neatly superimposable on one another.

Subsequent to this study two patients were given 1,200 ml of the same lot of intravenous fat emulsion on a single day; both had moderately severe febrile reactions. The next day following another infusion of the fat emulsion one of these had an even greater temperature rise and other untoward symptoms. Three months earlier he had suffered a severe reaction to long-term infusion of large amounts of intravenous fat emulsion.[1] When 22.7 per cent

TABLE II

	Greasy taste	Head-ache	Nausea	Feels warm	Aching arm	Dizzi-ness	Poor appetite	Good appetite
Control								
22 studies in 12 patients								
Number of reactions	0	0	0	0	0	0	1	9
Number of patients reacting	0	0	0	0	0	0	1	9
I. V. dextrose								
46 infusions in 12 patients								
Number of reactions	0	1	3	1	2	14	11	28
Number of patients reacting	0	1	2	1	2	7	5	9
I. V. fat emulsion								
27 infusions in 9 patients								
Number of reactions	11	5	2	3	4	3	2	16
Number of patients reacting	6	3	2	2	3	3	2	8

intravenous glucose was infused daily for one week to this patient, no fever developed.

The second patient had not previously received intravenous fat emulsion. After the temperature rise on the first infusion, there were no temperature rises for three weeks during which period he received 1,200 ml of lipomul daily. After three weeks of these large daily infusions this patient also suffered a severe reaction.[1] The experiences of these two patients suggest that the febrile response is due to a specific property of intravenous fat or a personal idiosyncrasy of certain subjects.

The blood data revealed few abnormalities over the period of study. Seven subjects had a high eosinophil count on a differential smear. Most of these counts were high prior to intravenous fat infusion and were not elevated further by intravenous fat.

Six out of nine patients followed for a period of two weeks had small weight gains, one remained constant, and two lost weight. Each day the patients were asked about their appetites, also, whether they suffered from headaches, dizziness and so forth. The incidence of these minor reactions is indicated in Table II. In addition to questioning subjects about their appetites, the amounts they had eaten for lunch were noted. The high incidence of good appetites and reports of definite hunger are surprising in view of the extra calories the patients had received during the morning infusions.

SUMMARY

Forty-six daily infusions of 22.7 per cent dextrose were given in a four-day period to 12 healthy men. The following week, nine of these subjects each received three daily infusions of a 15 per cent fat emulsion. The dextrose and fat emulsion infusions were approximately equal in caloric content and caloric rates of infusion. There were no febrile responses. The temperature patterns of the control days, dextrose infusion days and fat infusion days were substantially the same.

ACKNOWLEDGMENTS

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The Effect of Isonicotinic Acid Hydrazide and Vitamin B_6 on Glutamic-Oxalacetic Transaminase Levels in Whole Blood

By MARTIN SASS, M.S. AND GERALD T. MURPHY, M.D.

GROUP A served as a control and was selected at random from a large group of medical and surgical patients. Selection of patients was limited by the exclusion of cases with myocardial infarction, cirrhosis of the liver or hepatitis. Group B consisted of patients from the Chest Service of this hospital, all of whom had been receiving INH (300 mg/day) for at least 30 days. Group C consisted of nine men and four women volunteers selected from laboratory and ward personnel. All of these subjects were in good health, of average income, with presumed average dietary habits. In contrast to Group A, Group C was not fed from the hospital kitchen. Two additional groups of patients (D and E) who had never before received INH were selected at random from the Chest Service. Group D was placed on INH therapy (500 mg/day) and Group E received INH (500 mg/day) plus pyridoxine (25 mg/day).

Whole blood transaminase activity was measured at the start and at suitable intervals throughout the 6 to 12 week experimental period. Complete hematologic studies were done on these patients at the start, middle and end of the study. At the end of this experimental period, three patients in each group, D and E, received a tryptophan load test.[10]

The last group (Group F) consisted of selected patients with the lowest blood transaminase levels achieved after the administration of INH (500 mg/day) for 6 to 12 weeks. These patients continued to receive the same dose of INH plus supplementary multivitamin tablets without pyridoxine. Blood transaminase activity was measured at suitable intervals.

Laboratory Methods: Blood was collected in dry oxalate (Heller and Paul) and a 1:20 dilution was prepared in distilled water. This dilution of hemolyzed blood was centrifuged at 2500 rpm for 15 minutes to remove cell stroma. The clear supernatant was analyzed on the same day. In several cases, when necessary, the hemolysate was stored for 2 to 4 days at −20° C prior to analysis. The stability of the transaminase enzyme under these conditions is well established.

A modification[6] of the spectrophotometric procedure of Karmen and associates[7] was used for the estimation of glutamic-oxalacetic transaminase. This procedure is much more sensitive than that used by other investigators and does not present any of the reported difficulties[3,4] encountered with the use of the older procedure of Tonhazy, White and Umbreit.[8]

For this assay 0.2 to 0.3 ml of a 1:20 dilution of whole blood was analyzed. Readings were taken in a Beckman DU spectrophotometer, one unit representing a change in optical density of 0.001 O.D. units per minute, in a total volume of 3.0 ml under the conditions specified. All operations were carried out at room temperature. The temperature coefficients developed by Steinberg and Ostrow[9] were used to correct the values to a temperature of 25° C.

Lactic dehydrogenase (L.D.H.) levels were determined by an enzymatic technic based on the oxidation of reduced coenzyme I in the interconversion of pyruvic to lactic acid.[10]

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The concentration of hemoglobin in the dilution of blood analyzed was determined by spectrophotometric estimation of oxyhemoglobin at 540 mμ using a Beckman DU spectrophotometer. This instrument was standardized for hemoglobin determination by the manometric procedure of Van Slyke.[11] All other hematologic data were obtained by standard technics.

Studies in the pyridoxine-deficient state have been less revealing. In monkeys, vitamin B_6 deficiency produced through dietary restriction resulted in a reduction of blood transaminase levels below those of control animals.[3] No such differences have been reported in humans in whom pyridoxine deficiency is not so easily established. In one study of pregnant women whose response to tryptophan loading was characteristic of pyridoxine deficiency, blood transaminase values did not differ from those of non-pregnant controls.[4] To our knowledge, no other studies have been reported on this problem.

Recent reports indicate that the administration of isonicotinic acid hydrazide (INH) in high doses can produce a syndrome in humans suggestive of pyridoxine depletion.[5] In animals, INH has been used to produce a presumably more severe pyridoxine deficiency than can be produced by dietary means or by the administration of desoxypyridoxine.[2] These observations suggested the study of blood glutamic-oxalacetic transaminase (G.O.T.) levels in patients receiving INH in routine tuberculosis therapy.

EXPERIMENT

Subjects: Six groups of patients were studied in all. Three of these (Groups A, B and C) were studied to determine whether differences in whole blood G.O.T. levels existed in individuals receiving INH as compared with individuals not receiving this drug.

Pyridoxine or its metabolites are known to act as a prosthetic group for transaminase enzymes in animals.[1,2] While this relationship is presumably true in humans, evidence for this is indirect. Recent investigations in normal subjects[3] and in pregnant women[4] have indicated that supplementation with pyridoxine results in a significant elevation of blood transaminase activity. When supplementation is discontinued there is a slow return of blood enzyme activity to pre-administration levels.

RESULTS

The data in Table I indicate that the mean whole blood G.O.T. level of patients receiving 300 mg INH per day (Group B) is lower than that of the control series of hospital patients (Group A). Surprisingly enough, the mean blood transaminase level in non-hospitalized volunteers (Group C) is almost identical with that of Group B rather than with that of Group A, as might be expected.

TABLE I

Glutamic-Oxalacetic Transaminase and Lactic Dehydrogenase Activity in the Blood of Patients with and without Prolonged INH Therapy* (Mean ± S. E.)

	G.O.T.		L.D.H.
Group	Units/ml	Units/100 mg Hb	Units/mg Hb
A (Control)	723 ± 25 (23)	526 ± 20	154 ± 3.1 (12)
B (INH)*	550 ± 38 (19)	390 ± 26	152 ± 1.0 (16)
C (Volunteers)	590 ± 29 (13)	420 ± 6	157 ± 1.7 (13)
Significance (P)			
A vs. B	<0.01	<0.01	0.62
A vs. C	<0.01	<0.01	0.69
B vs. C	0.57	0.62	0.48

* INH therapy consists of 300 mg/day for a period of at least 30 days (numbers in parentheses refer to number of patients).

"P" values (Table I) indicate that the differences between Groups A and B and Groups A and C are highly significant. These differences did not change appreciably when the transaminase units were calculated on the basis of hemoglobin content indicating that variations in cell count, as reflected by hemoglobin content, did not account for the differences observed. All subsequent G.O.T. values are reported in units per 100 mg hemoglobin.

Blood lactic dehydrogenase levels (L.D.H.) of the three groups were also studied to

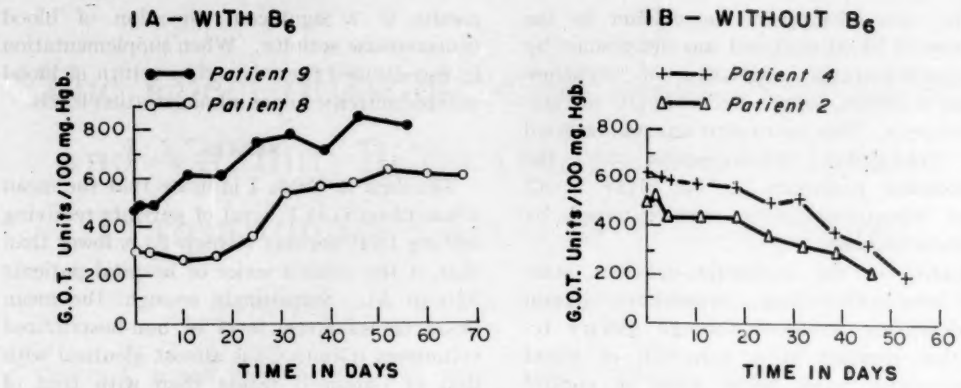

Fig. 1. Blood G.O.T. levels during INH therapy.

determine the specificity of the presumed effect of INH administration (Table I). The high "P" values indicate that there are no significant differences among the three groups.

Serial Studies: The blood G.O.T. levels during the experimental period of INH and vitamin B_6 administration are presented in Figure 1 for two representative patients in each of groups D and E. The increase in G.O.T. level during INH plus vitamin B_6 therapy (Fig. 1A) is marked beginning within 10–15 days after the start of pyridoxine administration. The decrease in G.O.T. activity observed during INH therapy alone, on the other hand, is limited and does not occur until 20–25 days of INH therapy at a level of 500 mg/day.

The transaminase values for each patient in groups D and E at the beginning and end of the experiment are presented in Table II. The differences between the mean pre- and post-therapy values for each group have been found to be statistically significant in contrast to the difference between the mean G.O.T. values for the two groups at the start of therapy. Plasma G.O.T. levels in all patients were within the normal range and could not influence whole blood transaminase levels significantly.

At the end of this experimental period three patients in each group were selected for a tryptophan load test. Twenty-four-hour urine specimens were collected on each patient for control levels. At the end of this period,

TABLE II

Change in Transaminase Activity in Whole Blood of Patients on INH Therapy with and without Vitamin B_6* (units/100 mg Hemoglobin)

	Patient	Pre-therapy	Post-therapy	Per cent change
Group D INH therapy	1	625	435	−31
	2	510	210	−59
	3	375	280	−25
	4	605	345	−43
	5	360	315	−12
	6	385	380	—
		Mean 475	330	−31
Group E INH and vitamin B_6 therapy	7	510	750	+47
	8	305	705	+130
	9	475	830	+75
	10	495	655	+32
	11	375	610	+62
		Mean 432	710	+69

* Therapy: 25 mg vitamin B_6/day and/or 500 mg INH/day over a period of from 5 to 11 weeks.

10 g DL-tryptophan* were administered by mouth and another 24-hour-urine collection was obtained. All urines were analyzed for xanthurenic acid[12] and the results are presented in Table III. They indicate that none of the patients in either group exhibited the marked increase in xanthurenic acid excretion indicative of pyridoxine deficiency.[13]

The mean hematologic values for each of the two groups at the beginning and end of the

* Generously supplied by Merck, Sharpe & Dohme, Inc.

TABLE III

Xanthurenic Acid Excretion after Tryptophan Load Test

Patient	Therapy	Xanthurenic acid mg/24 hours	
		Control*	Post-tryptophane
2	INH	29.8	48.0
3	INH	20.4	55.0
4	INH	28.8	34.4
8	INH and B_6	—	30.4
10	INH and B_6	16.4	1.9
11	INH and B_6	25.0	27.0

* 24-hour urine collection prior to administration of 10 g DL-tryptophan.

experiment are presented in Table IV. Since the hemoglobin content of the blood was used as a reference in calculating the G.O.T. level, any changes in the hemoglobin concentration per red cell (MCHC) could exaggerate changes in G.O.T. These changes did not occur. The significance of the apparent macrocytosis indicated by the elevated mean corpuscular volumes (MCV) is not apparent at the present time.

In order to evaluate the effect of vitamins other than pyridoxine, four additional patients (Group F) were studied. This group of patients had been receiving INH (500 mg/day)

TABLE IV

Hematologic Indices before and after Experimental Period

	MCV μ^3	MCH $\mu\mu g$	MCHC %	RBC *mill/mm³*	Hb *g/100 ml*	Hct %
Group D Pre-therapy	102	31.6	31.3	4.79	15.1	49
Group D Post-therapy	102	30.9	30.8	5.07	15.3	50
Group E Pre-therapy	101	29.1	28.9	4.66	13.5	47
Group E Post-therapy	100	30.2	30.4	4.70	14.2	47

MCV = mean corpuscular volume
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
RBC = red blood cells
Hb = hemoglobin
Hct = hemocrit

TABLE V

Transaminase Activity in the Blood of Patients on INH before and after Multivitamin Therapy* (Units/100 mg Hemoglobin)

Patient	Pre-therapy†	Post-therapy	% Change
2	210	210	0
4	280	200	−29
5	280	240	−16
6a	200	190	− 5
			Mean −12.5

* Two multivitamin tablets/day plus 500 mg INH/day for $6^1/_2$ weeks.

† Mean of two determinations one week apart.

for 6 to 12 weeks and were selected on the basis of low whole blood G.O.T. levels. They were continued on the same INH therapy and were given, in addition, a daily dose of two multivitamin* tablets without pyridoxine. G. O. T. levels were measured at intervals. The results (Table V) indicate that the administration of vitamins other than B_6 did not increase blood G.O.T. levels in the dosages employed.

DISCUSSION

In animals, INH has been demonstrated to produce a pyridoxine deficiency that is more severe than can be evoked by dietary restriction or by the administration of desoxypyridoxine.[2] In humans, peripheral neuropathy has been noted after doses of INH ranging from 24 mg per kg per day to as low as 3 to 5 mg per kg per day.[14] These toxic manifestations, amenable to pyridoxine therapy, are presumably due to the formation of an INH-pyridoxine complex,[5] effectively removing pyridoxine from the available pool for tissue utilization. The relative infrequency of overt clinical signs of toxicity at lower doses of INH, however, is no assurance that more subtle biochemical changes do not occur.

Our studies indicate that the administration

* Each tablet contains the following vitamins:

Vitamin A	— 5,000 U
Vitamin D	— 450 U
Thiamine	— 2 mg
Riboflavin	— 3 mg
Nicotinamide	— 20 mg
Ascorbic acid	— 75 mg

of INH results in a drop in blood transaminase activity despite the absence of clinical signs of toxicity. The mean G.O.T. activity in the blood of patients receiving 300 mg INH per day for a period averaging approximately four months (Table I, Group B) was found to be significantly lower than that of a control group on the same diet (Table I, Group A). This dosage level constitutes the usual regimen in the therapy of tuberculosis. More recently, some investigators have concluded that this dose is inadequate therapy and have suggested the use of a minimum of 8 mg per kg per day.[15–18] At approximately this level (500 mg per day) INH has been shown to depress blood transaminase activity within a period of from 6 to 11 weeks (Fig. 1B; Table II, Group D). This effect could be reversed by the administration of 25 mg pyridoxine per day (Fig. 1A; Table II Group E). Vitamins A and D, thiamine, riboflavin, nicotinamide and ascorbic acid were ineffective in the doses employed (Table V).

The significance of measurements of blood transaminase activity is certainly unclear at present. High doses of INH have already been shown to produce metabolic alterations indicative of pyridoxine deficiency.[5] With the dose used in this study (500 mg per day), tryptophan load tests at the end of the period of therapy were normal (Table III) in agreement with a previous report.[5] It is difficult, therefore, to avoid the suggestion that blood transaminase levels may serve as a more sensitive indicator of the adequacy or inadequacy of pyridoxine intake. The surprising finding that the mean G.O.T. activity of hospitalized control patients (Table I, Group A) exceeded that of non-hospitalized volunteers (Table I, Group C) could be a reflection of this relationship. It is certainly conceivable that the vitamin B_6 content of the routine, well-controlled hospital diet received by Group A was greater than that of the uncontrolled, non-institutional diet chosen by Group C. Unfortunately, no such measurements could be made.

Until further information becomes available on the meaning of the observed changes in transaminase activity during INH therapy, it would seem prudent to prevent their occurrence by the administration of small doses of pyridoxine whenever long-term INH therapy is necessary. The minimal dose of pyridoxine adequate to accomplish this has not as yet been determined. The amount used in this study has already been found to be effective in preventing peripheral neuritis in patients receiving 8 mg INH per kg per day.[17,18] This is probably in excess of that required to prevent changes in blood G.O.T. activity. It is expected that further study with smaller doses will indicate more precisely the amount of vitamin B_6 required to "neutralize" the effects of known amounts of INH.

SUMMARY AND CONCLUSIONS

The effect of INH and pyridoxine on whole blood glutamic-oxalacetic transaminase (G.O.T.) levels has been investigated. The mean blood G.O.T. activity of patients receiving 300 mg INH per day has been shown to be lower than that of control hospital patients but not significantly different from the mean of a group of normal, non-hospitalized subjects.

Serial studies at higher doses (500 mg INH per day) indicated that a significant drop in blood transaminase activity occurred after 6 to 11 weeks of therapy. At this time, tryptophan load tests were normal. These changes in blood transaminase activity were reversed by the administration of 25 mg pyridoxine per day and not by a group of other vitamins.

In view of these findings, it is suggested that blood G.O.T. activity may reflect the level of pyridoxine intake even before tryptophan metabolism becomes markedly abnormal. Until the actual significance of these changes becomes more clearly defined, it would seem advisable to combine the administration of pyridoxine with INH to prevent a depression in blood transaminase activity.

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Effect of Vitamin B_{12} on Growth-Retarded Children: A Review

By E. E. Howe, PH.D.

The effect of vitamin B_{12} on growth-retarded children has been a controversial subject since Wetzel[1] published his original observations. The accumulation of considerable new data prompts a reevaluation of the evidence.

Larcomb, Perry and Peterman[2] (Table I) have published the results of a carefully planned double-blind experiment carried out in the Ohio State Schools for the Deaf and the Blind. One hundred thirty-two children, six to ten years of age, were the subjects of the experiment which continued for seven months. Sixty children received a tablet containing 20 μg of vitamin B_{12} each day while 72 serving as controls received placebos identical in appearance. Neither children nor supervising teachers, nurses or physicians knew which children received the vitamin and which the placebo. When the groups were evaluated as a whole it was found that vitamin B_{12} most probably did exert a beneficial effect on growth, but the level of confidence fell slightly short of 95 per cent.

The report states[2] that Dr. John Tukey of Princeton University suggested a more detailed analysis of the data obtained. It was reasoned that since the test substance was a vitamin it should exert no effect on children who were not deficient in it. Since the subjects were chosen at random from a group receiving a well-balanced diet, it seemed reasonable to assume that not all were vitamin B_{12}-deficient, but that those who were had carried the whole group to the observed level of significance. If the deficient children could be identified and evaluated separately, more meaningful results might be obtained. Accordingly, the children were separated into three groups on the basis of their weight and height as related to age and conformation. Although the information was lacking in their original paper, it was learned by personal communication that roughly one-sixth were found to be definitely "underweight, two-thirds were of "normal" weight and one-sixth were "overweight." An evaluation of the underweight group revealed that there was less than one chance in one thousand that the observed increase in *weight* was fortuitous. No differences were found between the normal groups, but a statistically significant greater increase in *height* ($P<.05$) was found in the overweight group receiving vitamin B_{12} compared to the overweight group receiving placebo. Interestingly enough, the supervisors of the study, although unaware of the identity of the subjects receiving vitamin B_{12}, were in many cases able to identify them because of their improvement in alertness, behavior, and learning ability.

Recently Crump and Tully[3] (Table I) have reported a study involving 32 test subjects who received 25 μg of vitamin B_{12} and 10 mg of thiamine daily. These children were from 3 to 15 years of age and gave a clinical impression of undernutrition. In this experiment which was of 16 months' duration, the following results were obtained: ". . .50 per cent of the 32 children followed by the Wetzel grid technic evidenced quantitative gains which could be evaluated numerically. By grid analysis, 18 of these 32 children had demonstrated simple growth failure. Sixteen of these 18 children (88 per cent) responded by positive channel and auxodrome shifts, while the 14 children whose charts did not reflect growth failure showed no measurable response to the therapy."[3] Very early in the experiment there was a definite im-

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pression that the supplement was exerting a beneficial effect on the manageability, appetite, and activity of the children.

Jolliffe and his associates[4] carried out a careful study with 358 children consisting of boys between the ages of 6 and 12 and girls from 6 to 11 who were residing either in an orphanage in Rome or were attending an elementary school in Gaeta. One hundred seventy two (Table I) of these children who received 20 μg of vitamin B_{12} per day six days per week gained an average of 345 g more than a group of 179 who received a placebo. This experiment was of seven months' duration. The probability of this increase in weight gain occurring by chance is less than one in 1,000. There were no statistically significant differences in gains in height in the two groups.

Wetzel and collaborators[1,5] (Table I) have carried out studies in which 47 growth-retarded children have been treated orally with 10 μg of vitamin B_{12} daily. Before treatment the progress of these children had been plotted on the Wetzel grid for one and one-half to seven years and the records indicated a continuous growth failure. In these experiments which were of 6 to 16 weeks duration, 28 of the subjects responded to the administration of the vitamin by increases in rate of height and weight gain. Again the probability of this effect occurring by chance is reported to be less than one in 1,000.

In this experiment also the personnel of the medical and teaching departments noted improvement in behavior, attitude, and scholastic work.

Wetzel and his collaborators (Table I) also conducted a greatly extended study involving 236 children and have reported equivalent results although the details of this investigation have not been published.

Wetzel's work has been criticized for a variety of reasons, including lack of proper controls and failure to control psychologic factors. It would appear that the former criticism may be unjustified, since the development of each child had been plotted for a sufficient length of time before administration of the vitamin to allow him to act as his own control. In a later communication[6] the possibility of lack of control of psychologic factors seems to have been ruled out.

Chow[7,8] (Table I) has carried out experiments with both chronically ill and normal children. Forty-five inmates of a convalescent home suffering from such chronic illnesses as rheumatic heart disease, simple malnutrition or anemia, minor physical defects or mental retardation were the subjects of an experiment lasting three months. Twenty-one children received 10 to 25 μg of vitamin B_{12} daily and 24 untreated children served as controls. The difference between the increases in mean weight of the two groups (53.0 oz) was very highly significant statistically ($P<0.001$).

In a second study with children (Table I) chosen at random in a foundling home, 25 μg of vitamin B_{12} were administered daily to 9 out of 18 subjects of one-half to three years of age. The experiment continued for 24 weeks at which time the treated children had gained on an average 18 oz more than the control group. This difference is statistically significant ($P<0.05$). In experiments discussed further on in this paper it was shown that most infants do not respond to vitamin B_{12}. Chow's work is especially valuable in showing that by the time children have reached the age of two to three years some deficiencies may exist.

Quite recently Grüninger and his associates[9] (Table I) have conducted a study involving 145 growth-retarded children of preschool and school age. Administration of 10 to 15 μg of vitamin B_{12} caused a highly significant (99.9 per cent confidence level) increase in rate of weight gain of school children. Each child was subjected to a two to three week-control period before administration of the vitamin. It is questionable whether the control period in this study was of sufficient duration. No response to the vitamin was observed in a small group of 16 growth-retarded children of preschool age.

Wittich,[10] (Table I) has studied the effect of giving 5 to 15 μg of vitamin B_{12} daily for 13 months to 34 school children while 16 similar children served as controls. He has reported a highly significant effect of the vitamin on the rate of gain in height, weight and bone growth as measured by x-ray. In addition these

TABLE I

Ref. No.	Investigators	Location	Duration	Status of Subjects	Age	Test	Control	Vitamin B_{12} Dosage μg	Results
2	Larcomb, Perry and Peterman	Ohio State Schools Deaf and Blind	7 mo	Normal except for sight and hearing	6–10	60	72	20	Weight gain in thin significant ($P < 0.001$). Height gain in obese significant ($P < 0.05$). No difference in normal
3	Crump and Tully	Philadelphia	15.7 mo av	Clinical impression of undernutrition	3–15	32	Each child his own control for av of 28 months prior to treatment	25	88% of 18 in growth failure responded to treatment
4	Jolliffe *et al.*	Italy	7 mo	Immates of orphanage or school, not otherwise selected	6–12	170	188	20	Significant weight gain ($P < 0.001$)
1	Wetzel *et al.*	Cleveland, Ohio	6–16 wk	Children from families of high economic level in growth failure	Elementary and junior high school children	47	Each child his own control. Progress recorded for 1.5–7 yr	10	28 responded by increased rate of weight and height gain
5						236			Similar results although details not yet reported
7 8	Chow	Baltimore	3 mo	Chronically ill children in a convalescent home	6 mo to 12 yr	21	24	10–25	Weight increase statistically significant ($P < 0.001$)
7 8	Chow	Baltimore	24 wk	Residents in a foundling home	1½ to 3 yr	9	9	25	18 oz quota weight gain of treated group statistically significant ($P < 0.05$)
9	Grüninger *et al.*	Bad Dürrheim, Germany	Not recorded	Retarded, preschool and school children	3–10	86, 10 yrs 55, 6–10 yrs 16, 3–6 yrs	Each child served as own control during 2–3 wk period before treatment	10–15	Increased weight gain in school children significant ($P < 0.001$)
10	Wittich	Göttingen	13 mo	School children	5–12	34	16	5–15	Statistically highly significant increase in height and weight. Also highly significant increase in bone growth as measured by x-ray
11	Montoye *et al.*	Lansing, Michigan	7 wks	Boys in state correctional institution	12–17	16	16 placebo 19 no treatment	50	Prevention of weight loss in thin subjects

TABLE I—*Continued*

Ref. No.	Investigators	Location	Duration	Status of Subjects	Age	Test	Control	Vitamin B_{12} Dosage μg	Results
12	O'Neil and Lombardo	Omaha, Nebraska	4–12 wks	Malnutrition of undetermined origin	6 mo to 8 yr	32	—	25*	28 improved in general health, weight gain and appetite
13 14	Bidault	Paris, France	6–12 mo	Malnourished children	4 mo to 12 yr	52	—	25	Gain in height, weight and appetite
16	Scrimshaw and Guzman	El Salvador	25–31 mo	Malnourished growth failure, high incidence of parasitic infestation	School age	17	14	20	No significant effect on weight. Moderate effect on height
16	Scrimshaw and Guzman	Guatemala	11–28 mo	Malnourished growth failure, high incidence of parasitic infestation	School age	56	43	20	With B_{12} plus snack; some effect on weight, little on height compared to group receiving snack only. With B_{12} alone; rate of gain exceeded controls
15	Scrimshaw *et al.*	Guatemala	18 mo	Malnourished growth failure, high incidence of parasitic infestation	Preschool and school age	Total of 228 school children in both groups Preschool:		20	No differences in average monthly gains in height and weight
						43	46		
17	Benjamin and Pirrie	London, England	8 wk	Students at open-air schools, high percentage suffering from lung disorders	School age	418	413	10	No differences in gains in height or weight
22	Spies	Alabama	16 mo	Chronically malnourished. Diet deficient in calories and other essentials	4–14	9		22	No increase in rate of growth. Increased appetite, vigor and cooperation
23	Mackay *et al.*	Rural Jamaica	1 yr	Marginal malnutrition 70% of NRC recommended allowances for proteins and calories	School age	133	134	65	No increase in rate of growth

* In addition, 15 μg I.M., 2 to 6 injections at two-week intervals were administered.

children showed a remarkable increase in their mental functions as indicated by standard tests. Particularly noticeable was the increase in appetite.

At a state correctional institution in Lansing, Michigan, Montoye and his associate[11] (Table I) conducted a study on 16 boys 12 to 17 years of age who were given 50 μg of vitamin B_{12} daily for a period of seven weeks. The effects on height, weight, and performance in the Harvard Step Test were noted. Sixteen boys received placebos and nineteen controls received nothing. No effects of the vitamin were observed when all boys were considered, but in the thin boys loss of weight due to a competitive sport (half-mile race three times a week) was less than in those receiving placebos.

Thirty-two children from six months to eight years of age were treated by O'Neil and Lombardo (Table I).[12] These children all suffered from malnutrition of unknown origin. For the most part they received 25 μg of vitamin B_{12} daily by mouth and in addition from two to six intramuscular injections of 15 μg of the vitamin, usually at two-week intervals. Improvement in general health, growth rate and appetite was observed in all but four cases. Especially noteworthy were the observations that injections of vitamin B_{12} appeared to relieve several cases of severe diarrhea.

Bidault[13,14] (Table I) administered 25 μg of vitamin B_{12} by mouth daily to 52 malnourished children ranging in age from 4 months to 12 years. In almost all cases these children were reported to respond with increased gains in weight, height and appetite.

Scrimshaw, Tandon and Pérez[15] (Table I) recently have reported that 20 μg of vitamin B_{12} daily for 24 months produced no increase in growth rate of 43 Guatemalan children. In an earlier publication[16] the INCAP investigators had reported that the vitamin did produce a moderate gain in height in children both in El Salvador and in Guatemala despite the fact that the subjects were almost surely deficient in vitamin A and probably in other vitamins. In addition, these Central American children were almost all suffering from infestation with intestinal parasites, the effect of which on growth and development is not easily estimated. It seems likely that a deficiency of vitamin B_{12}, if present under these conditions, would not be the growth-limiting factor. This study indicates that Guatemalan children under existing environmental and nutritional conditions probably do not benefit from supplementation with vitamin B_{12} alone.

Benjamin and Pirrie[17] (Table I) reported that in a study of eight-weeks duration, involving a total of 497 boys and 334 girls, administration of 10 μg of vitamin B_{12} five times a week failed to produce a significant gain in height or weight. These children attended day open-air schools and were admitted for a variety of reasons. This study might be criticized on the basis of its short duration (eight weeks). However, since other investigators have observed a response in comparable periods, this shortcoming is not severe. A much more serious criticism is that a high percentage of these children were suffering from illnesses that undoubtedly were retarding their development and that could not be expected to be corrected by vitamin B_{12}. Thus a total of 348 children (42 per cent) were suffering from asthma, bronchitis, and bronchiectasis. The reasons for admission of the remaining subjects were undisclosed.

Three studies with premature infants by Downing,[18] Rascoff *et al.*,[19] and Finberg and Chow[20] have yielded definitely negative results. These infants did not respond to the vitamin and probably are not deficient in it. This can be explained readily by analogy with animal studies in which it has been shown that mothers transmit a considerable supply of vitamin B_{12} to their offspring, who subsequently may require a long time on a deficient diet to become depleted. More recently, Chow and collaborators[21] have shown that the concentration of vitamin B_{12} of the blood of the fetus is as much as three times that of the mother at the time of parturition.

Using the Wetzel technic, Spies[22] (Table I) investigated the effect of 30 to 75 μg of vitamin B_{12} daily on nine children receiving inadequate diets. None responded by increased growth although increased appetite, vigor, and cooperation were observed. There is, by the author's own statement, no question but that other de-

ficiencies also (including one of calories) were present, thus eliminating the probability of a growth response from the vitamin under test since other deficiencies could have been limiting.

Recently from a large scale study involving Jamaican children,[23] (Table I) it has been reported that supplementation with vitamin B_{12} was without effect. The subjects of this study were receiving about 70 per cent of the NRC recommended allowances for proteins and calories. Clinical examination presented a picture of marginal malnutrition. Here again, food essentials other than vitamin B_{12} probably were limiting. The authors suggest that positive results of supplementation with vitamin B_{12} might be observed in a population more chronically and severely undernourished than that in Jamaica. It would seem, however, that vitamin B_{12} is more likely to be the limiting factor in a population in which the protein and caloric intake is adequate.

DISCUSSION

The investigations described above are of varying degrees of importance and significance. Some are not well controlled and standing alone would be at best suggestive, but when taken as a whole, they offer strong supporting evidence for the view that vitamin B_{12} deficiency probably is the limiting factor in the growth retardation of some children. It is noteworthy indeed that in all experiments in which no response to supplementation with vitamin B_{12} was obtained there appear to be plausible explanations for this lack of response.

From a consideration of the results obtained in the experiments cited, the following conclusions seem justified:

(1) Infants, both premature and full term, will not respond to vitamin B_{12}, presumably because they are not deficient. They have a sufficient carry-over of the vitamin from their mothers. This carry-over, coupled with that contained in their normal diet, prevents the appearance of a detectable deficiency for an indeterminate period.

(2) A certain percentage of other children in this country appear to respond to vitamin B_{12}. This suggests that they may have been deficient in this vitamin.

(3) In countries in which the diet is grossly inadequate, other factors are likely to be more limiting than vitamin B_{12}. In countries such as Italy, in which the diet is more satisfactory but is deficient in animal foods, vitamin B_{12} deficiency is more likely to be the limiting factor.

The following question is valid: How, in this country of high-animal food intake, can a deficiency of vitamin B_{12} exist? To answer this question we must know the requirement for the vitamin and also its content in the American diet.

Use of radiolabeled vitamin B_{12} has shown that many patients with pernicious anemia are able to absorb no measurable amount of the vitamin from the gastrointestinal tract. Such people offer ideal material for studying the quantitative requirement of the vitamin. It is questionable whether such clear-cut results can be obtained with any other vitamin.

Several investigators,[24–28] have carried out studies designed to establish the quantity of vitamin B_{12} required when administered parenterally to maintain patients with pernicious anemia in remission. There is apparently considerable individual variation since quantities of from 0.3 to 2.0 μg per day have been reported. However, there appears to be rather general agreement that one μg per day is the approximate requirement. By use of radioactive vitamin B_{12}, Glass and his associates[29] have found in three normal subjects that the oral ingestion of 5 μg of vitamin B_{12} resulted in an hepatic uptake equivalent to that obtained by the parenteral administration of 1.10 ± 0.16 μg. Swenseid *et al.*[30] by means of a fecal excretion method have calculated an average absorption of 1.65 μg of vitamin B_{12} after the oral administration of 5 μg of the substance. The individual variation, however, was great; the calculated quantity absorbed in 15 tests ranging from 0.7 to 2.5 μg of the vitamin. These observations point to a daily requirement of the order of 5 μg but also emphasize that some individuals require a great deal more than others. This fact may be of importance in explaining the occurrence of vitamin B_{12} deficiencies within a group of individuals receiving very much the same diet.

Jolliffe and Peterman[31] have reported

recently that even a very good diet may furnish no more than 6 μg of the vitamin daily, much less than originally was estimated. A more economical diet may supply only 2 μg. If this is so, it might be anticipated that some persons will develop deficiencies.

A second important factor involved may be variability of absorption. Vitamin B_{12} has the largest molecular weight of any essential metabolite that is absorbed unhydrolyzed from the gastrointestinal tract.* The body has a special mechanism for its absorption. Many factors such as the bacterial flora, presence of parasites or deficiency of intrinsic factor, may decrease its absorption. In fact, some of the hematologic effects observed with antibiotics well may be due to elimination of bacteria that inhibit the absorption of the vitamin.

Certain "stress" factors may also cause an increased demand for vitamin B_{12} and thus contribute to the deficiencies observed. It is known from animal studies that such factors as infections, cold, thyroid and cortisone stress substantially increase the requirement for vitamin B_{12} and other water-soluble vitamins.[32–34] It is not unlikely that psychologic disturbances may also have a similar effect.

Although none of the investigations reviewed above indicate that vitamin B_{12} stimulates greater than normal growth, it has been suggested that rapid growth may not be altogether desirable[35,36] and the experiments of McCay are cited in support of this contention. It must be remembered that the beneficial effect of growth retardation on longevity observed by McCay[37] was brought about by caloric deprivation, which might be expected to produce no biochemical lesions such as have been encountered with vitamin deficiencies. It may be argued however, that a rapid rate of growth may not in itself be important. Nevertheless, it is difficult to believe that a "normalizing" influence, such as that observed in the experiments of Larcomb *et al.*[2] is not desirable.

Perhaps even more important than the effect on growth, but much more difficult to appraise objectively, is the reported effect of supplementation with vitamin B_{12} on behavior, alertness, and learning ability. The supervisors in double blind experiments usually were able to distinguish the children receiving the vitamin from those receiving the placebo because of improvement in these traits.

More detailed, carefully controlled clinical experiments are clearly indicated to give an unequivocal answer to the question of the prevalence and importance of vitamin B_{12} deficiency in children in this country. In the meantime, the existing data appear to be sufficiently impressive to warrant testing the effect of vitamin B_{12} administration on retarded children.

* Immunologic studies indicate that some proteins may be absorbed unchanged, but this is probably of little nutritional significance.

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Effect of Intrinsic Factor on Absorption of Vitamin B_{12} in Healthy Individuals

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VARIOUS investigators have demonstrated that intrinsic factor causes the absorption of orally administered vitamin B_{12} in pernicious anemia patients.[1–8] The effect of added intrinsic factor on absorption of vitamin B_{12} by normal individuals who secrete intrinsic factor has been the subject of relatively fewer reports. Swendseid *et al.*[9] found that administering intrinsic factor concentrates (from hog stomach mucosa) or normal human gastric juice led to a decrease in absorption of vitamin B_{12} in three of four normal individuals. Chow *et al.*[10] used ninety-one young healthy males and demonstrated that a crude intrinsic factor preparation inhibited absorption, whereas a highly purified preparation and a different crude preparation increased absorption of vitamin B_{12}. Investigation of the effect of a variety of intrinsic factor preparations[11] revealed that of those tested many generally available preparations inhibit while a few augment absorption of vitamin B_{12} by normal individuals.

In patients with pernicious anemia, increasing the dosage of certain intrinsic factor preparations to two or four times the minimum amount effective in producing remission was reported to inhibit absorption of vitamin B_{12}.[4,5] An intrinsic factor preparation known to be inhibitory at certain levels with normal subjects[10,11] was found at the same levels to increase absorption of vitamin B_{12} in patients with pernicious anemia.[6,7]

In the above experiments[9–11] the intrinsic factor preparations were given in relatively large doses and suspended or dissolved in water with radioactive vitamin B_{12}. This differs from the usual method of administration of vitamin B_{12}, intrinsic factor and other supplements for oral therapy of anemia. The present experiments were designed to investigate the effects of intrinsic factor and vitamin B_{12} as combined in vitamin-mineral capsules with other substances.

METHODS AND MATERIALS

For the determination of absorption of vitamin B_{12} the urinary excretion method of Schilling[2] was used. The subjects were residents of a state mental institution. They were in good physical health and were not receiving any other medications at the time of the test. The hematocrit values and vitamin B_{12} serum levels of these patients were all within the normal range. The patients, whose ages ranged from 24–40 years, were housed in one large ward and received identical diets.

The patients were randomly allotted to groups consisting of from six to thirteen subjects. In each experiment one group was given capsules containing the intrinsic factor preparation (0.5 daily oral dose* per capsule) and the following other ingredients: radioactive vitamin B_{12}, 10 μg (0.17 μc); folic acid,

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* We have defined a daily oral dose (DOD) of intrinsic factor as the minimum amount which causes satisfactory clinical improvement and an increase of about one million in red blood cell count in 21 days when given with 10 μg of vitamin B_{12} to a patient with pernicious anemia in relapse. Acceptance of the results obtained with a combination of one DOD and 15 μg of vitamin B_{12} or less by the U.S.P. Antianemia Advisory Board would permit reference to such a combination as a U.S.P. oral unit of vitamin B_{12} plus intrinsic factor.

1 mg; ascorbic acid, 50 mg; and $FeSO_4 . 2H_2O$, 192 mg per capsule. A second group which served as the control received capsules with the same ingredients except intrinsic factor. Each subject received three capsules per day, one at 7, 8 and 9 a.m., followed by a flushing injection of 1 mg of unlabeled vitamin B_{12} at 10 a.m. after which urine was collected for 24 hours. A second flushing injection was then given and urine collected again for 24 hours. Radioactivity measurements were made on urine aliquots up to one liter using a specially designed beaker.[12]

Intrinsic factor concentrates were prepared by extraction of hog gastro-intestinal tissue with 2 per cent NaCl and precipitation with $(NH_4)_2SO_4$ as described elsewhere[11,13,14] and were similar to "crude AS fraction."[13] The various preparations were from different batches made by the same process.

RESULTS AND DISCUSSION

The results of seven consecutive experiments are summarized in Table I. The urinary excretion of radioactive vitamin B_{12} in the individual subjects of a typical experiment (Experiment No. 1) is presented in Table II. The data were submitted to Dr. F. W. Wilcoxon for statistical analysis. When the number of patients in each group did not exceed ten, exact probabilities were obtained from tables published by the Mathematical Centre, Amsterdam,[23] after the values had been ranked[24] through both groups. In other cases the normal approximation was used with standard deviation of rank total equal to: $\sqrt{\frac{\bar{T}n_1 n_2}{6}}$ where $\bar{T}n_1$ is the expected rank total for the n_1 group, n_2 is the number of patients in the other group, while 6 is a constant.

TABLE I

Effect of Intrinsic Factor on Urinary Excretion of Vitamin $B_{12}Co^{56}$ by Normal Persons

Exp. No.	Patient group	Number of patients in group	Intrinsic factor concentrate in capsules	Average urinary excretion of vit. $B_{12}Co^{56}$ in 48 hours	Probability (P)
			Preparation No.	mμg.	
1	I	6	—	977	0.265
	II	7	182	1162	
2	III	6	—	990	0.314
	IV	7	182	1062	
3	II	6	—	1038	0.294
	I	6	87–3	1129	
4	IV	8	—	985	0.422
	III	6	87–3	1013	
5	V	13	—	814	0.258
	VI	12	87–2	849	
6	VI	13	—	711	0.215
	V	13	98–1	808	
7	VII	13	—	916	0.312
	VIII	13	635	1017	
				Combined Probability	0.23

TABLE II

Urinary Excretion of Vitamin $B_{12}Co^{56}$ in Normal Individual Subjects*

Without intrinsic factor		With intrinsic factor	
Subject	Excretion mμg in 48 hours	Subject	Excretion mμg in 48 hours
S.M.	902	J.A.	1022
J.T.	1070	F.B.	1249
J.T.	1371	J.B.	1170
D.V.	688	M.B.	892
E.W.	770	M.D	982
J.W.	1058	R.D.	1456
		D.H.	1366

* Experiment No. I.

The deviation of a rank total from the expected value $\bar{T}n_1$ was divided by the standard deviation and the probability obtained from Normal Tables. The expected total is the average rank times the number of items in the group. The average rank is $\frac{n_1 + n_2 + 1.}{2}$

The probabilities were combined according to Fisher[25] and are included in Table I.

Although the combined probabilities, $P = 0.23$, show only slight significance, intrinsic factor preparations administered in capsules together with vitamin B_{12} caused a small increase in the urinary excretion of vitamin B_{12} in seven consecutive experiments. Probably the reason that the effect of exogenous intrinsic factor was small was the fact that the absorption without intrinsic factor was high. Absorption without intrinsic factor in these experiments is about twice as high as reported by Chow *et al.*[10] using 50 μg of vitamin B_{12}.

Probably of greater importance was the lack of inhibition of absorption in contrast to reports of experiments with other intrinsic factor preparations.[10,11] Vitamin B_{12} serum levels have been reported to be decreased in persons who are iron-deficient,[15] elderly[16,17] or pregnant.[18–20] It is, therefore, desirable that measures be taken to assure the absorption of vitamin B_{12} by patients in these categories.

It has been reported that the absorption of vitamin B_{12} from the gastrointestinal tract of individuals with normal gastric function is limited. Glass *et al.*[21] and Swendseid *et al.*[9] have both found that the upper limit of absorption is 1.5 μg even after oral doses of 50 μg of the vitamin. This emphasizes the importance of non-inhibition or of augmentation of vitamin B_{12} absorption. Judging from the observed ratio that the amount of vitamin B_{12} absorbed is three times the 24-hour urinary excretion value or two times the 48 hour value,[22] the average amount of vitamin B_{12} absorbed by patients receiving intrinsic factor in this study was 2.0 μg. This compared closely with the upper limit of 1.5 μg reported by Glass *et al.*[21] and Swendseid *et al.*[9]

TABLE III

Effect of Intrinsic Factor on Vitamin B_{12} Serum Levels

No intrinsic factor μμg/ml	With intrinsic factor μμg/ml
Initial vitamin B_{12} serum level	Initial vitamin B_{12} serum level
240 ± 18.6	237 ± 25.8
at 6 weeks	at 6 weeks
248 ± 25.2	388 ± 37.4
at 12 weeks	at 12 weeks
239 ± 20.4	420 ± 31.5

± Standard deviation of the mean.

Capsules with essentially the same formula, but with nonradioactive vitamin B_{12} and with and without intrinsic factor were administered daily over a 12-week period to two groups of 13 elderly patients. Vitamin B_{12} serum levels were determined at 6 and 12 weeks. The results are shown in Table III. The increase in serum levels and hence the increase in absorption of vitamin B_{12} which was stimulated by intrinsic factor, was highly significant. $P = <.001$ at 6 and 12 weeks.

SUMMARY

Capsules containing several hematinic agents including intrinsic factor and vitamin B_{12} were administered to clinically healthy males. The intrinsic factor concentrate studied did not inhibit absorption of vitamin B_{12} as has been reported for some other preparations, but caused a slight increase in absorption of the vitamin, in seven consecutive experiments as measured by the urinary excretion tests. In a separate experiment the capsules containing intrinsic factor administered over a period

of 12 weeks caused a marked and statistically significant increase in vitamin B_{12} serum levels.

ACKNOWLEDGMENT

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Absorption of Vitamin B_{12} Enhanced by D-Sorbitol

By BACON F. CHOW, PH.D.,* PAUL MEIER, PH.D.,* AND SPENCER M. FREE, JR., PH.D.†

INTRINSIC factor concentrate has long been the only substance known to increase absorption of vitamin B_{12} from the gastrointestinal tract. In recent papers,[1-3] however, we described our findings that some substance or substances in an experimental lipotropic elixir enhanced vitamin B_{12} absorption in man and rats[2] and produced serum vitamin B_{12} levels significantly higher than could be expected from an oral preparation containing no intrinsic factor concentrates. This paper describes the series of studies by which it was established that vitamin B_{12} absorption is increased by D-sorbitol, a crystalline, hexahydric alcohol, $CH_2OH.(CHOH)_4CH_2OH$, used as a moisture stabilizer.

METHOD

The elixir under study contained, in each 5 cc:‡

Vitamin B_{12}, crystalline	8.34 μg
Riboflavin	0.6 mg
Pyridoxine hydrochloride	2.0 mg
Niacinamide	7.0 mg
Betaine, anhydrous	700.0 mg
Choline dihydrogen citrate	150.0 mg
Inositol	150.0 mg
Ferric pyrophosphate (soluble)	35.0 mg
Caffeine citrate	65.0 mg (1 gr)
Alcohol	15%
D-sorbitol	q.s.

In addition, the elixir contained coloring and flavoring. Since the elixir contained a great number of components, we had to consider that the enhancing effect might be due to the interaction of a number of chemicals with possible synergistic effects. We decided, however, to search first for one or two factors. If more than two factors appeared to be involved, the problem would be considered too complex for our present test methods.

The "factors" considered were themselves combinations of components, as follows: (a) alcohol and caffeine; (b) D-sorbitol; (c) vitamins-riboflavin, niacinamide, and pyridoxine hydrochloride; (d) betaine, choline dihydrogen citrate, and inositol. Other ingredients, color, flavor, and ferric pyrophosphate, were considered to be a part of the base and were not investigated separately but were included in all experimental preparations.

Since we were looking for no more than two factors, we studied all possible combinations of factors taken two at a time, plus a control and the full formula in an exploratory study. As shown in Table I, this required an experiment using eight different formulae.*

Absorption of vitamin B_{12} was measured in male volunteers among inmates of a state prison. The men were clinically healthy and free from acute infectious diseases or metabolic disturbances at the time of the study. All volunteers worked in the prison shops and obtained their food from the same kitchen. They had a well balanced diet with adequate nutritional sources of vitamin B_{12}. Their daily ration contained at least 0.18 kg of meat and an average of 0.8 of an egg and 100 ml of milk each day in addition to the amounts of eggs and milk used in making breads and desserts.

Vitamin B_{12} absorption was estimated using

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‡ Supplied by Smith, Kline & French Laboratories, Philadelphia, Pennsylvania.

* This experimental design constitutes a $1/2$ replication of a 2^4 factorial design.[4]

the technic of Schilling.[5] In all tests,* isotopic dilutions of vitamin B_{12} were made so that the specific activity of the final dilution was equal to 50 μc/mg. Each volunteer was given a solution of radioactive vitamin B_{12} containing 50 μg of the vitamin in 10 ml of a test solution. The beaker containing the radioactive vitamin B_{12} was rinsed two times with 10 ml of the test solution and three times with 10 ml of distilled water. The total fluid intake for each man was thus equal to 60 ml. Two hours after the volunteer ingested the test solution, he was given an intramuscular dose of 1 mg of unlabeled vitamin B_{12} to "flush" out the radioactive vitamin B_{12}.

A 24-hour urine collection was then made with the precautions described by Chow *et al.*[6] During the first 12 hours in which urine was collected, the volunteers were kept in one room and were supervised carefully. They were permitted to sleep in their dormitory during the night, but in the morning the men were again required to stay in one room in order that the 24-hour urine collection could be completed under close supervision.

A new method of comparing several treatments was developed for this study because the common procedure is too often unwieldy and quite variable. Under ordinary procedures, individual doses are pipetted from bulk supplies of each of the test solutions. Men are treated in order of their arrival and all doses of one solution are administered before the next solution is given. Consequently, whenever a large number of men are studied at one evaluation, the last man receives his dose of test solution long after the first man has been treated. Since doses are given on an empty stomach, one may question whether observed differences in response are caused by differences in the solutions or by differences in the time of administration. In addition, so much time may be required to administer the solutions that it is difficult to control the timing of the two-hour intramuscular "flushing" injection of vitamin B_{12}.

The method used in this study greatly reduces these problems. Test solutions were prepackaged in individual 30 ml doses. Each of the different test solutions was given once, in random order, before any one solution was given a second time. This procedure was repeated as often as necessary. Each evaluation was thus balanced for time effects and all doses were measured exactly and without haste. Each bottle of solution was assigned a code number which designated the order in which it was to be given, and which enabled us to keep the study "double-blind."

The code number identification was carried through the laboratory work, thus providing a randomized plan for the whole investigation. In particular, single regimens were not treated as one group so that any variation in laboratory work would not be credited to differences between the regimens. The code was not broken until all laboratory work was recorded for each code number, and the results were tabulated by specific treatments. The final results were balanced for time effects and included only random laboratory variation and biologic variation. It was found that variability between subjects was materially reduced by using this method of pre-packaging test solutions.

Additional work showed that when this technic was used, we needed only eight men for each test solution to detect meaningful differences in response to treatment. It was also found that an optimum size for any one day's experiment was 24 to 32 men. This limited size of the experiment helped to control the timing for the flushing, intramuscular dose of vitamin B_{12}.

RESULTS

The results of this study are shown in Tables I–IV. From the first series of studies (Table I) it appeared that none of the combinations reached the level of absorption given by the full formula; however formulas which contained vitamins were closer to the results of the full formula than those without vitamins. It was therefore decided to investigate vitamin B_{12} absorption from solutions containing riboflavin, niacinamide and pyridoxine. Since formula 7 which contained D-sorbitol produced

* The radioactive vitamin B_{12} labeled with Co^{60} and Co^{58} used in the Schilling tests was obtained from Merck & Company.

TABLE I

Schilling Test. Exploratory Study

Formula no.	Mixture tested*	Number of subjects	Average vitamin B_{12} μg (in 24 hr)
1	Control (water alone)	9	545
2	Lipotropics, alcohol and caffeine	8	549
3	D-sorbitol, alcohol and caffeine	9	586
4	Lipotropics, D-sorbitol	6	431
5	Vitamins, alcohol and caffeine	7	759
6	Vitamins, lipotropics	8	735
7	Vitamins, D-sorbitol	7	833
8	Full formula	8	900

* Basic formula: color, flavor, ferric pyrophosphate.
Standard deviation: 250.
Significant difference between treatment averages: 250.

TABLE II

Schilling Test. Individual Vitamin Study

Mixture tested*	Number of subjects	Average vitamin B_{12} $m\mu g$ (in 24 hr)
Control (water)		565 ± 50 ($P_{.05}$)
Riboflavin, Niacinamide	7	758
Riboflavin, Pyridoxine	8	582
Niacinamide, Pyridoxine	7	712
Riboflavin, Niacinamide, Pyridoxine	8	731

* Basic formula: 40% D-sorbitol.
Standard deviation: 159.
Significant difference between treatment averages: 159.

vitamin B_{12} levels nearest to those of the full formula, D-sorbitol was used in the base of all of these solutions.

The results of this study (Table II) showed that none of the preparations containing combinations of the vitamins (riboflavin, niacinamide and pyridoxine) enhanced absorption as much as the full formula did. However, the solution without niacin yielded somewhat lower vitamin B_{12} levels than did the others, although all vitamin solutions tested were higher than the control (50μcg of vitamin B_{12} in aqueous solution). These results seemed to indicate that either niacin was the active component or the enhancing substance was present in all four solutions tested.

TABLE III

Urinary Excretion. Effect of Niacinamide

Mixture tested*	Number of subjects	Average vitamin B_{12} $m\mu g$ (in 24 hr)
Control	8	579
Niacinamide	8	611
Niacinamide and D-sorbitol	7	764
Full formula	8	806

* Standard deviation: 154.
Significant difference between treatment averages: 154.

TABLE IV

Urinary Excretion. Confirming Effects of D-Sorbitol

Mixture tested*	Number of subjects	Average vitamin B_{12} $m\mu g$ (in 24 hr)
Control	9	581
D-sorbitol	8	852

* Standard deviation: 201.
Significant difference between treatment averages: 195.

It was decided to test the effect of niacin first. The scheme of the study and the results are given in Table III. The results indicate that niacin without D-sorbitol was not superior to the control, but when D-sorbitol was present, vitamin B_{12} absorption was close to that of the full formula. This study led us to question our tentative assumption that the active agent was a vitamin and suggested that D-sorbitol may have been the substance which enhanced the absorption of vitamin B_{12} in the elixir.

A fourth study was conducted to test whether D-sorbitol was the active agent. To this end, nine subjects were given 50 μg of radioactive vitamin B_{12} in an aqueous solution; whereas, the second group of eight subjects received 50 μg of radioactive vitamin B_{12} mixed with D-sorbitol. The results of this study are given in Table IV. They clearly indicate that D-sorbitol can enhance the absorption of vitamin B_{12} from the gastrointestinal tract (this effect of D-sorbitol has been noted also in similar studies in rats).*

* Greenberg, S. *et al.*, Smith, Kline & French Laboratories. Personal communication.

DISCUSSION

In spite of the limitations of the Schilling urinary excretion test as a measure of the absorption of vitamin B_{12}, it provided a convenient means for comparing the activity of various test substances. The precision of the measurements in this study was increased by testing the substances on an unusually homogeneous group of subjects: prisoner volunteers who received the same food and who could be carefully controlled. Moreover, by pre-packaging and randomizing test solutions, individual responses within each group were made comparable. These procedures not only saved time, but avoided much of the confusion which ordinarily arises when a large group of individuals is involved in a volunteer test. Furthermore, by administering the test solutions within a short period of time, the differences in physiologic reactions such as secretion of gastric juice or psychologic factors which may influence the absorption were minimized.

TABLE V

Control Observations

Number of subjects	Average vitamin B_{12} $m\mu g$ (in 24 hr)
9	546
9	545
8	579
9	581
8	575
8	577
5	580
5	607

The advantages of this procedure are best indicated by the constancy of excretion of radioactivity in the urine of the control subjects who received the solution at different times over a period of more than one year. The results are presented in Table V. The constancy of our control values enables us to interpret with fair confidence the small differences in the urinary excretion among the groups.

At the present time, we are not certain how D-sorbitol aids the absorption of vitamin B_{12} from the gastrointestinal tract. The enhancing effect may be physiologic or it may be the result of a chemical reaction between vitamin B_{12} and D-sorbitol. It may even be caused by a contaminant in the D-sorbitol.

Regardless of the mechanism, the finding that a simple chemical substance, not derived from the usual animal sources of intrinsic factor, can increase vitamin B_{12} absorption is of academic interest and practical importance. The observation suggests a new line of research into the mechanisms of vitamin B_{12} absorption and deficiency disorders. It also suggests further study which may lead to a means for improving vitamin B_{12}-therapy for pregnant, convalescent, geriatric, and other patients who may have a mild state of vitamin B_{12} deficiencies.

SUMMARY AND CONCLUSIONS

A multiple-component lipotropic elixir which enhanced absorption of vitamin B_{12} was analyzed to determine which of its many ingredients caused the increased absorption.

In order to analyze the elixir, the usual methods of studying the effects of nutritional substances upon human volunteers had to be improved. A new method was developed which has a number of statistical advantages in addition to improving the precision and reducing the time required to conduct such studies.

Study results showed that D-sorbitol, a widely used moisture stabilizer, substantially enhanced the absorption of orally administered vitamin B_{12}. This is the first substance other than intrinsic factor concentrate shown to have the ability to increase vitamin B_{12} absorption from the gastrointestinal tract.

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Studies on the Mechanism of Action of Vitamin B_{12} in Animal Nutrition

By B. CONNOR JOHNSON, PH.D.

EXTENSIVE reviews on vitamin B_{12} have recently appeared.[1,2] This paper will therefore be limited primarily to a discussion of our own studies on the metabolic functions of vitamin B_{12} using as experimental subject principally the pig, but also at times the chick, the rat, and various micro-organisms. From many laboratories numerous functions of vitamin B_{12} have been proposed. These include the involvement of vitamin B_{12} directly or indirectly in hematopoiesis (chemical level not known), transmethylation, purine and/or nucleic acid biosynthesis, methyl biosynthesis, sulphydryl synthesis or maintenance, CH_2OH reduction, formation of "functional form" of folic acid, carbohydrate metabolism, protein metabolism and fat metabolism. The extent of this list at least indicates the importance of this vitamin in metabolism.

PRODUCTION OF THE DEFICIENCY

In 1947 we started feeding a "soy-protein synthetic milk" ration to baby pigs (Table I). On this ration the animals died unless given an anti-pernicious anemia liver extract.[3] The deaths were shown to be due to vitamin B_{12} deficiency,[4,5] following the isolation of this vitamin by the Merck group.[6] Typically vitamin B_{12}-deficient baby pigs are shown in Figures 1 and 2. The weight gain response to various levels of vitamin B_{12} is illustrated in Figure 3. From this work, it appeared that the requirement of the baby pig for vitamin B_{12} was of the order of 0.6 μg/kg body weight if administered by intramuscular injection and about twice this, 20 μg/kg dry matter of the diet, when fed in the ration.[7] Weight data and liver vitamin B_{12} values from a typical baby pig experiment are illustrated in Table II.[8] After studying the gross symptoms of the deficiency in the young pig,[9,10] it appeared to us that, due to the lack of stores and the ease and rapidity of production of a severe vitamin B_{12} deficiency in this species in contrast to the nondepleted rat or chick, the baby pig would be a useful animal in which to study the function of this substance. Since carrying out this work on the baby pig, we have also produced reasonably satisfactory vitamin B_{12} deficiencies in nondepleted chicks and rats using the rations shown in Tables III,[11–13] and V[14] with the growth data shown in Tables IV and VI. Table VII presents hematologic data on the rats fed the soy-lactose diet.

TABLE I

Soy-Protein "Synthetic Milk" Ration for Baby Pigs. Composition of Synthetic Milk Ration Used to Produce Vitamin B_{12} Deficiency in the Baby Pig

Ingredient	%
α-protein	27.6
DL-methionine	0.4
Cerelose	32.3
Citric Acid	1.7
Lard	28.8
Minerals	9.2
Vitamins	+

Minerals, g/kg dry ration		Vitamins, mg/kg dry ration	
$Ca(OH)_2$	18.45	Thiamine	5
KOH	7.3	Riboflavin	10
NaOH	7.1	Pyridoxine HCl	10
$CaCl_2 \cdot H_2O$	14.3	Nicotinic acid	20
MgO	2.3	Ca pantothenate	40
KH_2PO_4	6.9	Folic acid	0.4
NaH_2PO_4	37.6	Biotin	0.1
$CuSO_4 \cdot 5H_2O$	0.018	2-methyl-1,4-naphthoquinone	2.0
KI	0.051		
$MnSO_4$	0.09	Vitamin A	15,000 IU
$ZnCl_2$	0.015	Vitamin D	1,540 IU
CaF_2	0.03	Vitamin E	100
Ferric citrate	1.7		

From the Laboratory of Animal Biochemistry, Department of Animal Science, University of Illinois, Urbana, Illinois.

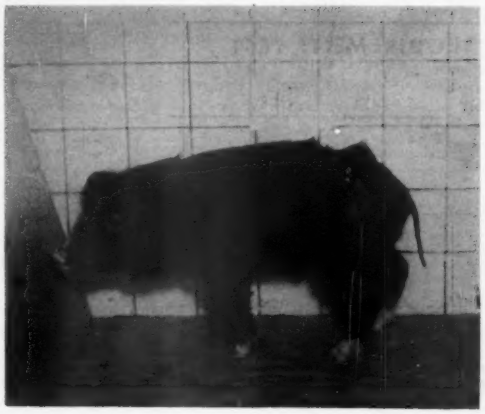

Fig. 1. Vitamin B_{12}-deficient baby pig, six weeks' old.

indicating even more strikingly the success of this ration in producing a vitamin B_{12} deficiency in the young rat. Thus, we have also been able to use these two species for metabolic function studies.

TRANSMETHYLATION

The possibility of vitamin B_{12} functioning in transmethylation has been proposed and we have studied it both in the intact animal by means of nutritional experiments and at the tissue level by means of *in vitro* studies.

TABLE II

Weight Gain and Liver Vitamin B_{12} Content of Normal and Deficient Baby Pigs in One Typical Experiment

Diet	Number of pigs	Weight gain, *kg*		Liver vitamin B_{12}* *mμg/g* fresh wt.
		4 weeks	5th week	
Plus vitamin B_{12}	5	8.26	3.59	135
Vitamin B_{12}-deficient	6	3.79	0.68	10
Vitamin B_{12}-treated†	4	2.30	2.12	37

* By *Ochromonas malhamensis* assay.[8]

† Given by injection a single dose of 50 μg per pig of vitamin B_{12} at the end of the 4th week.

Using the baby pig, we have demonstrated a requirement for choline of 0.1 per cent of the diet, on a diet containing 30 per cent Labco casein which supplied 0.8 per cent methionine, 2 per cent serine, 0.6 per cent glycine, and adequate vitamin B_{12}. At the 1.5 per cent methionine level no choline was required for the prevention of fatty livers or renal damage, thus demonstrating the ability of the normal pig to synthesize choline by transmethylation from methionine[15] as had been previously demonstrated for the rat and other species. Baby pigs were fed the vitamin B_{12}-deficient diet supplemented to contain 1.5 per cent methionine

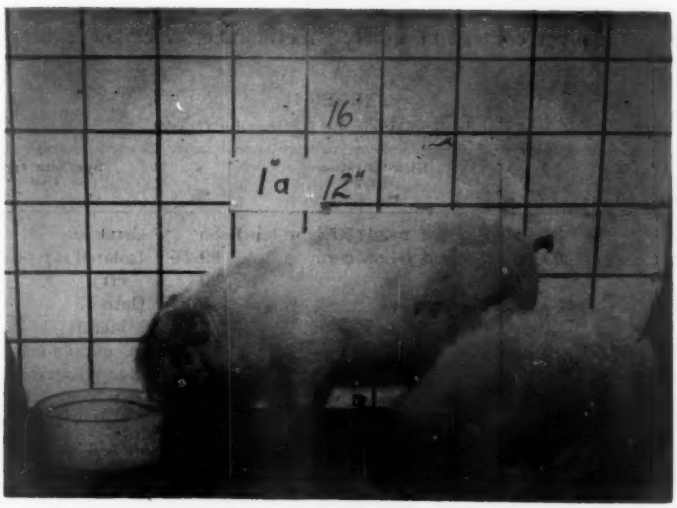

Fig. 2. Vitamin B_{12}-deficient and vitamin B_{12}-supplemented baby pig, four weeks old.

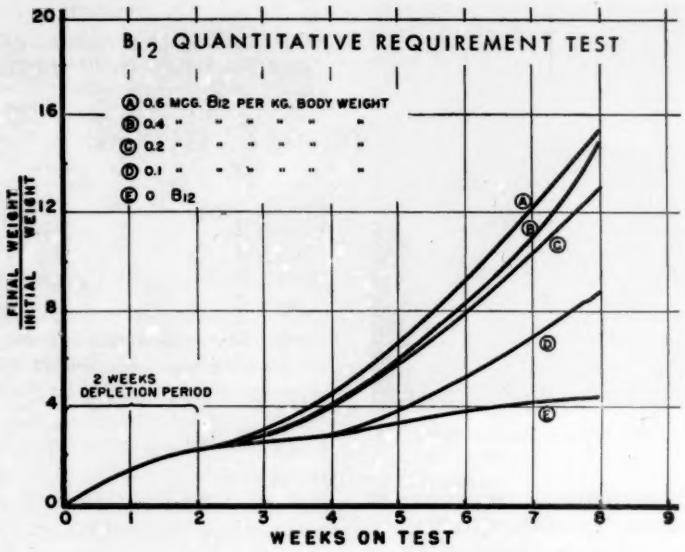

Fig. 3. The effect of various levels of injected vitamin B_{12} on the growth of baby pigs.

but no added choline and compared to the same diet supplemented with vitamin B_{12}. It was found that neither group of pigs shows the fatty livers of choline deficiency although the pigs on the vitamin B_{12}-deficient diet did develop typical and severe vitamin B_{12} deficiency symptoms.[16,17] This demonstrates clearly that vitamin B_{12} was not required *in vivo* for this transmethylation. The liver content of vitamin B_{12}, choline and urinary choline values for these pigs are given in Table VIII. The difference in body weight and liver vitamin B_{12} indicate the severity of the vitamin B_{12} deficiency induced. The data of Arnstein,[18] using C-14 methyl-labeled choline, showed clearly in the intact rat that vitamin B_{12} is not required for the transmethylation from choline to methionine, since the ratio of radioactivities

TABLE III

Vitamin B_{12}-Deficiency Rations for Chicks

High soybean meal ration Diet 1[11]	%	High-fat ration Diet 2[12]	%	Synthetic type diet Diet 3[13]	%
Ground yellow corn	33.95	Soybean meal (50% protein)	35	Cerelose	64
Soybean meal (50 per cent protein)	60.00	Ground yellow corn	39.56	Isolated soy protein (Drackett)	24
Dicalcium phosphate	3	Lard	20	Corn oil	6
Ground limestone	1	Minerals	5.34	Minerals	5.5
Vitamin A and D oil (3000 units A and 600 D)	0.3	Choline chloride	0.1	DL-methionine	0.3
Iodized salt	0.5	plus all vitamins except B_{12} (including folic acid but not PABA or inositol)		plus all vitamins except B_{12} (including folic acid but not PABA or inositol)	
Manganese sulfate	0.05				
Alfalfa meal	1.0				
DL-methionine	0.2				
plus riboflavin, calcium pantothenate, choline, and nicotinic acid					

TABLE IV

Results of the Use of the Various Vitamin B_{12}-Deficient Rations Given in Table III Fed to Non-Depleted Chicks (New Hampshire—Columbian Cross) (Weight in grams)

Ration (from Table III)	Weeks on diet	$-B_{12}$	$+B_{12}$ 50 μg/kg ration
(1) High-soybean meal	4	294	319
	9	936	1201
(2) High-fat	4	226 (42)*	296 (308)*
(3) Synthetic	4	376	435
	7	688	803
	15	1670	1850

* The values in ( ) are the liver vitamin B_{12} assays in mμg/g determined by *Ochromonas*.

TABLE VI

Results of the Use of the Two Vitamin B_{12}-Deficient Rations Given in Table V Fed to Weanling Male Rats (Gain in grams)

Diet (from Table V)	Days on diet	$-B_{12}$	$+B_{12}$*
Diet 1	42	187	208
Diet 2	33	98.6	134

* Diet 1, 50 μg/kg diet. Diet 2, 7.5 μg/rat/day, fed orally twice a week.

In order to study different transmethylation reactions, *in vitro* technics have been used.[20,21] In these experiments, homogenates were prepared from the livers of normal and B_{12}-deficient baby pigs, chicks, and rats. Using the method of Borsook and Dubnoff,[22] the transmethylation from methionine to guanidoacetic acid to form creatine was studied. In this procedure by the addition of ATP, folic acid, and glutamic acid, this transmethylation will take place in the methyl group of choline and of methionine after 31 days' feeding was the same for the vitamin B_{12}-deficient as for the vitamin B_{12}-normal animals. Stekol *et al.*[19] have shown for the intact rat that vitamin B_{12} deficiency has no effect on transmethylation to choline or creatine.

TABLE V

Vitamin B_{12}-Deficient Rations for Rats

Diet 1[12]		Diet 2[14]	
Drackett 220 (soy) protein	20	Full-fat soya flour	72
DL-methionine	0.4	Lactose	22
Cerelose	69.37	Salts (Hubell, Mendell and Wakeman, 1937)	4
Corn oil	6.0	Skade	2
Minerals 446	4.0	Vitamin supplement	
Choline chloride	0.1		
Vitamin A	2,000 IU } per 100 g		
Vitamin D	200 IU } diet		
α-tocopherol	10 mg }		
Thiamine hydrochloride	2.5 mg		
Riboflavin	1.6		
Ca pantothenate	4		
Nicotinic acid	10		
Pyridoxine HCl	0.6		
Biotin	0.06		
Folic acid	0.4		
2-methyl-1,4-naphthoquinone	0.1		

Skade (fat-soluble vitamin solution)		Vitamin supplement/kg of diet in mg	
Vitamin A conc.	2×10^6 IU	Inositol	220
Calciferol	0.25 mg (10^5 IU)	Ca-D-pantothenate	100
α-tocopherol acetate	14.2 g	Niacin	100
Menaphthone	100.0 mg	PABA	75
Archis oil	to 100 g	Thiamine	30
		Riboflavin	30
		Pyridoxine	8
		Folic acid	1
		Biotin	0.2

TABLE VII

Blood Picture in Vitamin B_{12}-Deficient and Normal Rats (Soy-Lactose Diet)

Vitamin B_{12} status	Red cell count *million/cu mm*	White cell count */cu mm*	Hemoglobin *g/100 ml*
$+B_{12}$ (50 μg/kg/diet)	7.5	6,000	13
$-B_{12}$	5.5	4,000	9

under aerobic conditions. Table IX shows the lack of effect of prior vitamin B_{12} deficiency on the ability of pig liver homogenates to carry out this transmethylation.

ported a depressing effect of vitamin B_{12} deficiency on this transmethylase. Table X shows that in the case of the pig there is no effect of vitamin B_{12} deficiency nor of the *in vitro* addition of vitamin B_{12} on the activity of this transmethylase in baby pig liver. Table XI shows that this is also true in the case of the normal and vitamin B_{12}-deficient chick. However, the data in Table XI do conform with those of Williams *et al.*[23] in showing a significant difference in transmethylase in the case of rat liver in vitamin B_{12} deficiency. In view of our data on the intact pig and on the

TABLE VIII

Growth, Liver Vitamin, B_{12}, Liver Choline, and Urinary Choline of Pigs

Group	Av initial *wt, kg*	Av 4-week *wt, kg*	Av liver choline		Av liver B_{12}		Av urinary choline excretion	
			$-B_{12}$ *mg/g*	$+B_{12}$* *fresh*	$-B_{12}$ *mμg/g*	$+B_{12}$* *fresh*	$-B_{12}$	$+B_{12}$* *mg/24 hr*
1 Positive Control ($+B_{12}$)	1.93	10.19	—	2.03	—	290	—	—
2 Basal	1.91	5.41	2.63	2.37	18.6	39.7	2.84	2.31

* Two pigs in Group 2 were given one dose of 50 μg of vitamin B_{12} by injection at 4 weeks. The $+B_{12}$ refers to these pigs in Group 2.

The second transmethylation studied was from betaine to homocysteine to form methionine. Williams *et al.*,[23] using rat livers, have re-

transmethylase of the pig and the chick, and in view of the work of Arnstein *et al.*[18] and of Stekol *et al.*,[19] we feel that vitamin B_{12} is not

TABLE IX

Creatine Formation by Normal and Vitamin B_{12}-Deficient Pig Liver Homogenates

Group	Number of pigs	Creatine formed μg *per g* liver per hr	
		No addition	+ vitamin B_{12}*
Normal	5	84 ± 7	—
Deficient	6	97 ± 17	95 ± 14
Deficient vitamin B_{12} treated†	3	75 ± 2	65 ± 6

1 ml of 20 per cent homogenate. Initial concentrations of reactants, guanidinoacetic acid 10^{-3} M, L-methionine 10^{-3} M, L-glutamic acid 10^{-3} M, and adenosinetriphosphate 1.5×10^{-3} M. Folic acid 100 γ per 6 ml of reaction mixture. All the solutions were prepared in Cohen and Hayano's sodium potassium phosphate buffer, pH 7.4; total volume, 6 ml; gas phase, oxygen; time, 1 hr; temperature, 38°.

* *In vitro* addition of vitamin B_{12}, 100 mμg per 6 ml of reaction mixture.

† A single dose of vitamin B_{12}, 50 γ per pig, was injected at the end of four weeks.

TABLE X

Transmethylase Activity of Normal and Vitamin B_{12}-Deficient Pig Liver Homogenates

Group	Number of pigs	Methionine formed γ *per g* liver per hr	
		No addition	Vitamin B_{12} + citrovorum factor*
Normal	5	296 ± 18	—
Deficient	4	293 ± 11	282 ± 8
Deficient Vitamin B_{12} treated†	4	309 ± 27	278 ± 23

2 ml of 16.7 per cent homogenate, 0.5 ml each of 0.5 per cent betaine hydrochloride (neutralized) and 1 per cent DL-homocysteine. All the solutions were prepared in Cohen and Hayano's sodium potassium phosphate buffer, pH 7.4. Total volume, 3 ml; gas phase, nitrogen; time, 3 hr; temperature, 38°.

* *In vitro* addition of vitamin B_{12} and calcium leucovorin, 50 mμg and 50 γ, respectively, per 3 ml of reaction mixture.

† A single dose of vitamin B_{12}, 50 γ per pig, was injected at the end of 4 weeks.

TABLE XI

Methionine Formation by Liver Homogenates of Various Species

Animal	Group	Number of animals	Methionine formed* γ *per g* liver per hr	
			SH	S—S
Rat	Normal	5	193 ± 16	61 ± 7
Rat	Deficient	5	141 ± 9 (P = 0.03)	50 ± 19
Chick	Normal	5	220 ± 19	80 ± 6
Chick	Deficient	5	270 ± 20	97 ± 10

* Experimental details are given in Tables IX and XII. SH = DL-homocysteine as the substrate. S—S = homocystine as the substrate.

TABLE XII

Methylation of Homocystine with Betaine by Normal and Vitamin B_{12}-Deficient Pig Liver Homogenates

Group	Number of pigs	Methionine formed γ *per g* liver per hr	
		No addition	Vitamin B_{12} + citrovorum factor*
Normal	5	121 ± 4	107 ± 6
Deficient	5	105 ± 8	—
Deficient vitamin B_{12}-treated†	4	106 ± 5	101 ± 1

1 ml of 16.7 per cent homogenate, 0.5 ml of 0.2 per cent betaine hydrochloride (neutralized), and 2.5 ml of 0.02 per cent homocystine. All these solutions were prepared in Cohen and Hayano's sodium potassium phosphate buffer, pH 7.4. Total volume, 4 ml; gas phase, nitrogen; time, four hr; temperature, 38°.

* *In vitro* addition of vitamin B_{12} and calcium leucovorin, 50 mμg and 50 γ, respectively, per 4 ml of reaction mixture.

† A single dose of vitamin B_{12}, 50 γ per pig, was injected at the end of four weeks.

TABLE XIII

B_{12} and Blood Glutathione (GSH) Baby Pigs

	Control B_{12}	$-B_{12}$	+ Pseudo B_{12}
Number of pigs	2	5	4
Blood GSH, mg/100 ml	21.6	13.2	13.2

directly involved in these transmethylations, and that the differences observed in the case of the rat must be due to some as yet unexplained difference in enzyme level (see later). In work with an *E. coli* mutant, Dubnoff[24] has indicated that vitamin B_{12} might be involved in the reduction of —SS— groups to sulphydryl groups. To study this, we examined the betaine transmethylase using homocystine as substrate. The results of this work with rat and chick liver homogenates are also given in Table XI and the data on pig liver homogenates are given in Table XII. As can be seen, no significant differences were observed between the normal and the vitamin B_{12}-deficient livers in their ability to carry out the overall reaction—homocystine reduction to homocysteine and methylation by betaine to form methionine. The addition *in vitro* of vitamin B_{12} or leucovorin had no effect on this transmethylation. It thus seems that vitamin B_{12} does not play a direct role in the reduction of —SS— to —SH. In the whole animal, however, Ling and Chow,[25] working with rats, reported that vitamin B_{12} deficiency results in a marked lowering of blood glutathione. As can be seen from Table XIII we have obtained similar data from the baby pig. The reason for these results is not apparent at the moment.

METHYL SYNTHESIS

Nutritional Studies—Pigs: Growth studies with rats by Bennett[26] and with chicks by Gillis and Norris[27] first indicated that vitamin B_{12} functions in the synthesis of methyl groups. We have studied this function both in terms of its being a physiologically important reaction *in vivo* and in terms of its possible demonstration by the use of C^{14}-labeled compounds. We thus investigated which precursors of the one-carbon unit require vitamin B_{12} for conversion to methyl groups.

The nutritional experiments were carried out with baby pigs, chicks, and rats. In the baby pig experiments,[28,29] the vitamin B_{12}-deficient soy-protein diet supplemented to contain 0.8 per cent methionine, but with no added choline, was fed to four groups of pigs as follows: group 1, 1 per cent glycine added and adequate B_{12}; group 2, 1 per cent glycine added and no B_{12}; group 3, no glycine added and adequate B_{12}; and group 4, no glycine added, adequate B_{12} and adequate choline added. All pigs were killed between the fifth and sixth week, when the group 2 pigs were severely B_{12}-deficient, and the livers were examined histologically and by chemical analysis for fat. It was

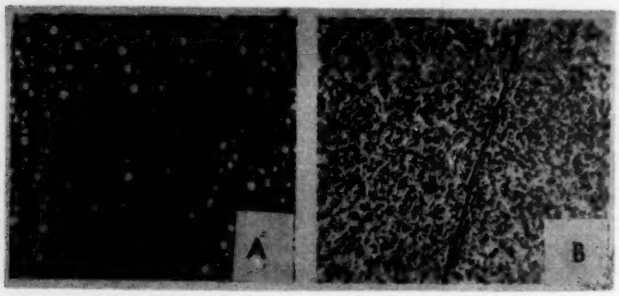

Fig. 4. The effect of vitamin B_{12} on the prevention of choline deficiency-fatty liver by glycine. Photomicrographs of liver sections of pigs at termination of experiment. Magnification 157.5. All sections were made by the paraffin method and stained with Harris' hematoxylin and cosin. All animals received 2.2 per cent glycine and no choline. A. Liver from pig receiving no B_{12}. B. Liver from pig receiving 0.8 μg B_{12}/kg body wt/day.

found that in the presence of vitamin B_{12} added glycine (group 1) prevented the fatty livers of choline deficiency (Fig. 4) while in the absence of vitamin B_{12}, or of added glycine (groups 2 and 3), fatty livers were obtained. These results indicate that vitamin B_{12} is required for the synthesis of methyl groups from glycine and that in the case of the baby pig this synthesis can be quantitatively extensive enough to be physiologically important.

A series of five similar experiments were carried out on the possibility of other compounds serving as choline precursors for the prevention of fatty livers in choline-deficient baby pigs.[30] The results of these experiments are summarized in Table XIV. It appears that serine is similar to glycine in serving as a choline precursor even in this type of nutritional experiment and that vitamin B_{12} is again required for the choline-methyl synthesis. Formate also appears to be lipotropic but no effect of vitamin B_{12} was apparent in these experiments. However, the C^{14}-formate experiments below show clearly that vitamin B_{12} is involved in methyl synthesis from formate in these pigs. In these limited experiments, histidine and leucine were inactive. In another nutritional experiment, choline deficiency in the presence of vitamin B_{12} was produced over a three-week period following which choline, serine, and glycine were administered therapeutically for seven days. The results of this experiment are given in Table XV. From all these experiments, it appears that both serine and glycine may be a physiologically important methyl group precursor in the case of the baby pig.

Isotope Studies—Pigs: These same pigs

TABLE XIV

Summary of Five Experiments on Effect of Various Possible One-Carbon Donors on the Liver Fat of Baby Pigs Fed a Choline-Low Diet

Supplement	Number of experiments	$+B_{12}$		$-B_{12}$	
		Number of pigs	liver fat	Number of pigs	liver fat
None	5	15	25.3	—	(26)*
0.1% choline	5	11	8.5	—	(8)*
1.4% DL-serine	3	10	17.4	9	25.1
0.4% Na formate	2	11	15.7	11	13.7
1% histidine	1	1	29.9	—	—
2% leucine	1	3	26.6	—	—

* Data taken from baby pigs on earlier experiments on transmethylation.

TABLE XV

Curative Effect of Choline and Choline Precursors Administered to Choline-Deficient (Three-Week Old) Baby Pigs (All Adequate in Vitamin B_{12})

Supplement (given for seven days)	Number of pigs	Liver fat %	Histology
None	6	28.8	Fatty
0.1% choline	3	7.3	Normal
1% DL-serine	1	6.5	Normal
1% glycine	3	11.4	Normal

have in many cases been given C^{14}-labeled "one-carbon" precursors four hours before sacrificing, and choline and methionine have been isolated from the carcasses of both the vitamin B_{12}-normal and the deficient pigs in order to study more directly the effect of the vitamin on methyl synthesis.

In Table XVI are given the results of the experiments[31] involving α-C^{14}-glycine. These data show very clearly (five fold increase) that vitamin B_{12} is required for the biosynthesis of methyl groups from the α-carbon of glycine. They also show that vitamin B_{12} is not involved in the metabolic pathway glycine → serine → ethanolamine (in contrast to the earlier report of Stekol, Weiss and Weiss[32]). In contrast to the report of Luck and Wilcox,[33] who were unable to demonstrate the formation of aminoethanol from glycine in the rat, we found (Table XVI) that a considerable proportion of the radioactivity of the glycine fed was trapped at the aminoethanol level by cold aminoethanol (0.1 per cent of the diet) which was fed to these pigs.[31]

Having demonstrated this marked requirement for vitamin B_{12} for methyl synthesis from the α-carbon of glycine, we next proceeded to

TABLE XVI

The Incorporation of Radioactivity from Glycine-C^{14} into Serine and Choline of Livers Activity* ($\times 10^2$)

Pig	Serine	Total	Choline† trimethyl-amine–moiety	Amino-ethanol moiety
Normal	916	857	178	679
Deficient	830	643	34	609

* Expressed as counts/min/μM glycine injected.

† The isolated crystalline derivatives were recrystallized to constant radioactivity.

TABLE XVII

Weight Gains (40 days) of Pigs Used to Study Effect of B_{12} on Methyl Synthesis from Serine and Formate

Pig number	B_{12}	Choline	C^{14} (40 μc/kg body weight injected)	Weight at 40 days *kg*
3-3	—	—	serine-3-C^{14}	6.9
9-1	+	—	serine-3-C^{14}	9.5
9-10	—	—	formate-C^{14}	6.0
9-9	+	—	formate-C^{14}	8.5

C^{14} experiments with β-C^{14}-serine and C^{14}-formate to clarify studies with these one-carbon precursors. Table XVII gives the weight data, showing the extent of vitamin B_{12} deficiency, of the baby pigs used in the serine and formate experiments, and the methyl incorporation data are shown in Table XVIII. In this

TABLE XVIII

Methyl Synthesis in the Pig from β C^{14}-Serine and C^{14}-Formate

Compound injected	B_{12} status	Activity of isolated compound (μc/mM)	
		Choline	Methionine
Serine-3-C^{14}	+	0.3160	0.108
	−	0.1122	0.039
Formate-C^{14}	+	0 2310	0.0742
	−	0.0692	0.0271

experiment, in addition to choline, methionine was also isolated by the Floyd-Lavine procedure as adapted by Weiss, Anderson, Hsu and Stekol.[34] The radioactivities in the isolated choline and methionine samples were determined by wet combustion and gas-counting in the Vibrating Reed Electrometer. The results show a clear-cut effect of vitamin B_{12} deficiency on the utilization of both formate-C^{14} and serine-3-C^{14} for methyl synthesis. The serine data are in contrast to the earlier results of Stekol *et al.*[32,35] for the rat but are in agreement with those of Arnstein[18] also for the rat. We feel, then, on the basis of our baby pig work, that vitamin B_{12} is required for methyl group synthesis not only from the α-carbon glycine but also from the β-carbon of serine and from formate.

Nutritional Studies—Chicks: We have also carried out both nutritional and tracer studies

TABLE XIX

Perosis as an Index of the Effect of Vitamin B_{12} on Methyl (Choline) Synthesis from Glycine*

Diet contains 0.68 per cent total methionine plus cystine			
Supplement		Perosis, %	
Choline %	Methionine %	No B_{12}	Plus B_{12}
none	none	90	90
0.02	none	50	10
none	0.3	90	90
0.02	0.3	40	10
0.05	none	20	0
0.05	0.3	0	0
0.20	none	0	0

* 2% added to all diets.

TABLE XX

Methyl Synthesis in the Chick and the Role of Vitamin B_{12}

Labeled compound injected	B_{12} status of bird	Radioactivity of compounds isolated μc/nM	
		Choline	Methionine
Me-C^{14}-methionine	+	0.2708	0.091
Me-C^{14} methionine	−	0.2968	0.099
$HC^{14}HO$	+	0.03	0.016
$HC^{14}HO$	−	0.2754	0.097
Serine-β-C^{14}	+	0.2030	0.062
Serine-β-C^{14}	−	0.1156	0.0402
Formate-C^{14}	+	0.0603	0.0221
Formate-C^{14}	−	0.0301	0.0098

Chicks were sacrificed 48 hr after administration of the labeled precursors. In each case, 30 μc/kg body wt of chick of labeled precursor was administered.

on the role of vitamin B_{12} in methyl synthesis using the chick as experimental animal. In the chick nutrition studies, perosis was used as index of choline nutriture. After some preliminary trials, we found that it was possible to demonstrate that glycine would aid in the prevention of perosis in the chick in the presence of vitamin B_{12} but not in its absence. The data are summarized in Table XIX and demonstrate not only the effect of vitamin B_{12} on choline synthesis (presumably from the 2 per cent glycine added to these diets) but indicate also that in the *chick* glycine may be a quantitatively more important precursor of choline than is methionine. Certainly it again appears that methyl synthesis can be of physiologic importance. Scott[36] had earlier reported similar results with regard to vitamin B_{12} and hock disorder in turkeys.

Isotope Studies—Chicks: In addition to these nutritional experiments, methionine-methyl-C^{14}, serine-β-C^{14}, formate-C^{14}, and formaldehyde-C^{14} have been given to vitamin B_{12}-deficient and vitamin B_{12}-normal chicks. These chicks were kept on experiment for 48 hours in each case in order to obtain uric acid collections, etc., and then sacrificed. From the carcasses, choline and methionine were isolated, as in the case of baby pigs. The data on methyl group labeling are summarized in Table XX. These results further confirm the lack of effect of vitamin B_{12} on transmethylation. They indicate also that vitamin B_{12} is not involved in the utilization of formaldehyde for methyl synthesis but that it is apparently playing an important role in some other pathway of formaldehyde utilization. The serine and formate data are in complete agreement with the C^{14} data obtained using the baby pig, showing again that vitamin B_{12} is required for methyl synthesis from these precursors. The results were apparent after 48 hours as they had been after four hours from the injection of precursor.

Rat Studies: In four rat experiments attempting to duplicate the baby pig nutrition experiments on methyl synthesis wherein glycine replaced a part of the methyl requirement in the presence of vitamin B_{12}, it appeared that the efficiency of methyl synthesis in the rat is not adequate[37] to protect rats from choline deficiency at any dietary methyl level which would produce a methyl deficiency in the absence of vitamin B_{12}. However, using tracers, the C^{14} studies of Stekol *et al.*[19] have indicated that vitamin B_{12} is required for methyl synthesis from glycine but not from serine or formate, while the studies of Arnstein *et al.*[18] have indicated that vitamin B_{12} is required for methyl synthesis from glycine, serine, and formate and that of these, serine is the principal methyl precursor.

Ochromonas Studies: Another approach to the problem of methyl synthesis and vitamin B_{12} function has been through the use of the organism *Ochromonas malhamensis* which has a

TABLE XXI

One-Carbon Precursors and Growth of *O. Malhamensis*

Supplement	% transmission	
	$-B_{12}$	$+B_{12}$
None	87	23
L-methionine 1.2 mg	71	19
Sodium formate 0.2 mg	87	80
Glycine 3 mg	86	13
DL-serine 3 mg	86	15
L-histidine 3 mg	84	17
Ethanolamine 1 mg	85	19
Sodium glyoxalate 1 mg	85	16

Cultures were grown at 30° C for four days in the dark with shaking in 2 ml medium and read after dilution to 9 ml.

TABLE XXII

One-Carbon Precursors and Reversal of Ethionine Inhibition of *O. Malhamensis*

Supplement	% Transmission Ethionine 0.7 mg	
	$-B_{12}$	$+B_{12}$
None	89	72
L-methionine 1.2 mg	89	21
Sodium formate 0.2 mg	88	80
Glycine 3 mg	88	14
DL-serine 3 mg	87	20
L-histidine 3 mg	86	44
Ethanolamine 1 mg	87	79
Glycolic acid 2 mg	89	75
Sodium glyoxalate 1 mg	86	87

Cultures were grown at 30° C for four days in the dark with shaking in 2 ml medium and read after dilution to 9 ml.

specific requirement for vitamin B_{12}. Although this organism does not have an absolute methionine requirement, growth is stimulated by the addition of methionine. Using this system, it was found that glycine and serine would replace methionine in the presence, but not in the absence, of vitamin B_{12}. The data for these experiments are given in Table XXI and confirm the general concept that, as in the animal, certain one-carbon precursors of methyl groups require vitamin B_{12} for methyl synthesis. Homocysteine plus choline does not replace methionine irrespective of vitamin B_{12} status, suggesting that the methyl groups are synthesized on the sulfur rather than elsewhere and then transferred to homocysteine.[38]

Further data of improved specificity were obtained by the inhibition analysis method. Marked growth inhibition of *Ochromonas* is brought about by the methionine antagonist, ethionine, and is completely reversed by methionine. For this purpose methionine can be replaced by certain one-carbon sources when vitamin B_{12} is included in the medium. A summary of some of these data is given in Table XXII. It appears that the most active methionine precursor is L-serine as was found by Arnstein and Neuberger[18] in the rat. Glycine and other methyl precursors are also active in replacing methionine for the reversal of ethionine toxicity. The response to homoserine is interesting and is possibly related to its metabolism to glycine.

To study directly the synthesis of the methyl group of methionine from glycine, *Ochromonas* cells were grown in the presence of α-C^{14}-glycine and homocysteine both in the presence and in the absence of vitamin B_{12}. These growing cell experiments showed that methionine is a metabolic product of the labeled glycine, labeled methione being found in the cell protein hydrolysates.[38] Cell-free preparations from *Ochromonas* cells grown with excess and inadequate vitamin B_{12} were next studied. Such cell-free preparations from vitamin B_{12} adequate cells were found to metabolize α-labeled glycine, the radioactivity appearing in at least 12 other compounds. One of these compounds was isolated and identified as methionine by repeated carrier crystallization as methionine sulfoxide.[39] Preparations from *Ochromonas* grown with limiting vitamin B_{12} were less able to metabolize glycine and produced no detectable C^{14}-methionine. In order to be sure that these differences were specific for vitamin B_{12} deficiency and not just due to the use of moribund cells, the cell-free preparations were repeated with cells deficient and normal with respect to thiamine and in both cases adequate with respect to vitamin B_{12}. Both these cell-free preparations were found to make labeled methionine from labeled α-C^{14}-glycine. Cell-free preparations from *E. coli* capable of methionine synthesis have also been described.[40]

Possible pathways of glycine metabolism include: (1) the formation of the hydroxymethyl group of serine from the α-carbon of glycine, possibly followed by its transfer to homocysteine and then its reduction to methyl, the transfer from serine to homocysteine possibly being by way of the "folic acid coenzyme"; (2) the oxidative-deamination of glycine to glyoxylic acid which then is decarboxylated to yield CO_2 plus an "active formaldehyde";[41] or (3) the formation of δ-aminolevulinic acid and its conversion to α-ketoglutaraldehyde and then to succinate plus "active formaldehyde."[42] Glyoxylic and glycollic acids, as well as δ-aminolevulinic acid, all failed to serve as methionine precursors in these experiments, and the C^{14}-glycine experiments failed to yield serine as one of the major products of metabolism. Thus, it seems that much more needs to be learned as to the metabolic pathways involved before the exact site of action of vitamin B_{12} in methyl synthesis can be determined. We feel that the cell-free preparations described will help in the future work we hope to do in this area.

PURINE BIOSYNTHESIS

Chick Uric Acid Synthesis: Certain vitamin B_{12}-requiring mutants of *E. coli* will grow as well on methionine as on vitamin B_{12}, indicating that methionine (methyl) synthesis is the only function of vitamin B_{12} for this organism.[43] On the other hand, we have found many times that a baby pig will die of vitamin B_{12} deficiency in the presence of excess methionine plus choline, proving that there must be other functions of vitamin B_{12} in the animal which are more vital than methyl synthesis. If one considers some of the lactic acid bacteria, it has been found that thymidine and other nucleotides will at least partially replace vitamin B_{12} for *Lactobacillus lactis* Dorner and *Lactobacillus leichmannii*. The possible role of vitamin B_{12} in nucleic acid biosynthesis has therefore been investigated.

TABLE XXIII

Degradation of Urinary Uric Acid Following Methionine-CH_3-C^{14} Injection*

Position	% of total uric acid activity
Carbon 6	1
Carbons 4 and 5	7
Carbons 2 and 8	92
Carbon 8	36
Carbon 2	56

* Sime, J. T. (1954), Ph.D. Thesis, Univ. of Illinois

In work on the metabolism of methyl-C^{14}-methionine by the chick, it was found that approximately half of the urinary C^{14} excreted by the chick following an intraperitoneal injection of the labeled methionine was in uric acid, the label occurring almost exclusively in positions 2 and 8, i.e., from methyl via the hypothetic "active one-carbon" precursor (Table XXIII).[44] It seemed to us then that uric acid formation in the chick should be an excellent tool with which to study purine biosynthesis in the intact animal and the effect of vitamin B_{12} deficiency on such biosynthesis. Normal and vitamin B_{12}-deficient chicks were given by intraperitoneal injection methyl-C^{14}-methionine, α-C^{14}-glycine, β-C^{14}-serine, C^{14}-formate, and C^{14}-formaldehyde. By means of a prior surgical operation, urine uncontaminated by feces could be collected, and this was usually done over a 48-hour period, from each bird. The overall data for glycine, serine, and formate are given in Table XXIV. As can be seen, the specific activity of the isolated uric acid was about 1.6 times higher in the normal than in the vitamin B_{12}-deficient bird following C^{14}-formate administration. A similar difference in uric acid activity between normal and vitamin B_{12}-deficient birds is found following serine-β-C^{14} administration. However, this difference is not large compared to the differences observed in methyl synthesis, and there is much less difference in the case of glycine. When methyl-C^{14}-methionine was administered and uric acid labeling followed with urine, the data given in Table XXV were obtained. In the vitamin B_{12}-normal chicks, the highest specific activity was found in the zero to four-hour sample, while in the vitamin B_{12}-deficient birds the maximum labeling occurred much later, in the 8 to 12-hour sample, and the total incorporation was only about one-fifth of that in the vitamin B_{12}-normal chicks.[45,46]

These data seem to indicate that vitamin B_{12} is more intimately involved in the utilization

TABLE XXIV

Incorporation of Glycine-2-C^{14}, Serine-3-C^{14}, and Sodium Formate-C^{14} into Urinary Uric Acid of Normal and Vitamin B_{12}-Deficient Chicks*

Compound	Amount injected μc	Chick	Weight g	Uric acid† Specific activity μc/mM	
				Actual	Corrected‡
Glycine-2-C^{14}	40	Normal	691	1.074	1.074
(2 mc/mM)	40	Deficient	742	0.951	1.021
Serine-3-C^{14}	40	Normal	803	0.579	0.579
(1 mc/mM)	40	Deficient	688	0.458	0.391
Na Formate-C^{14}	100	Normal	1,228	2.727	2.727
(4.45 mc/mM)	100	Deficient	1,201	1.695	1.660

* Eight-week-old birds were used for the glycine and serine groups and 13-week-old for the formate group.

† Isolated from the 48-hr urine collections of the formate and serine groups and 30-hr urine of the glycine-injected birds, since the collection after 30 hr was contaminated with the feces.

‡ S. A. of the deficient samples were adjusted to correspond to the respective body weights of the normal birds.

of the methyl of methionine as a one-carbon precursor than in the incorporation of such an "active one-carbon compound" into uric acid. Studies with formaldehyde-C^{14}, shown in Table XXVI, gave a very different picture but one which corresponds exactly with the formaldehyde to methyl results. The highest specific activity was found in the uric acid excreted during the first four-hour period by both the normal and the vitamin B_{12}-deficient birds, but this time the specific activity of the uric acid from the deficient birds was about three times that from the normal birds. Thus, vitamin B_{12} does not appear to be involved in the utilization of formaldehyde for purine or for methyl synthesis but for some other reaction which is reduced in the deficient birds. Some of the increase in specific activity of uric acid following C^{14}-formaldehyde administration may be due to the somewhat reduced total uric acid excretion due to vitamin B_{12} deficiency. These totals were determined by isotope dilution analysis and are given in Table XXVII.

Allantoin Labeling—Rats: It has been shown that the methyl group of methionine also serves as a precursor of allantoin—the nucleic acid purine excretion product—in the rat.[47] The effect of vitamin B_{12} deficiency on the incorporation of various one-carbon precursors into allantoin has been investigated. Methyl-C^{14}-

TABLE XXV

Incorporation of Methionine-CH_3-C^{14} into Urinary Uric Acid of Normal and Vitamin B_{12}-Deficient Chicks

Urine collection hr	Uric acid Specific activity μc/mM	
	Normal	Deficient
0– 4	1.611	0.037
4– 8	1.291	0.223
8–12	0.441	0.520
12–16	0.444	0.369
16–26	—	0.322
26–38	0.404	0.180
38–49	0.263	0.146

Two normal and two vitamin B_{12}-deficient 15-week old chicks were each injected 100 μc of 0.42mc/mM DL-methionine-CH_3-C^{14}. The average weight of the normal and deficient birds were 1,850 and 1,671 g, respectively.

TABLE XXVI

Incorporation of Formaldehyde C^{14} into Urinary Uric Acid of Normal and Vitamin B_{12}-Deficient Chicks

Urine collection hr	Uric acid Specific activity μc/mM	
	Normal	Deficient
0– 4	5.801	14.013
4– 8	0.328	0.749
8–12	0.120	0.270
12–16	0.088	0.275
16–26	0.064	0.192
26–38	0.065	0.138
38–49	0.071	0.071

Two normal and two vitamin B_{12}-deficient 28-week-old chicks were each injected with 33 μc/kg body wt of formaldehyde-C^{14}. The average weights of the normal and deficient birds were 2,525 and 2,250 g, respectively.

TABLE XXVII

HC^{14}HO Experiment—Total Uric Acid Excreted (by Isotope Dilution Assay)

Urine collection *hr*	Uric acid excreted, *mM*	
	Normal	B_{12} deficient
0– 4	2.88	2.06
4– 8	3.51	2.02
8–12	2.59	0.99
12–16	—	—
16–26	9.16	2.68
26–38	5.37	1.76
38–49	3.74	2.24

methionine, α-C^{14}-glycine, β-C^{14}-serine, and C^{14}-formate were given daily over a seven-day period to vitamin B_{12}-deficient and vitamin B_{12}-normal rats, and the urine collected for the total period from each rat. The allantoin was then isolated and counted for each animal.

TABLE XXVIII

Ability of Rats on Vitamin B_{12}-Free and Complete Diets to Incorporate Various Precursors into Allantoin

Experiment	Precursor	Treatment	Dose *c/m/day*	Specific activity* of allantoin *c/m/mg*
4	2-C^{14}-glycine	B_{12}-free diet	1.45×10^6	789
	2-C^{14}-glycine	Complete diet	1.45×10^6	773
5	C^{14}H$_3$-methionine	B_{12}-free diet	1.65×10^6	576
	C^{14}H$_3$-methionine	Complete diet	1.65×10^6	472
6	β-C^{14}-formate	B_{12}-free diet	2.0×10^6	5,473
	β-C^{14}-formate	Complete diet	2.0×10^6	4,777
7	β-C^{14}-serine	B_{12}-free diet	1.87×10^6	2,130
	β-C^{14}-serine	Complete diet	1.87×10^6	2,313

* Corrected to 200-g rats. Three rats per group.

The data are summarized in Table XXVIII. As can be seen, no effect of vitamin B_{12} deficiency was found on the incorporation of any of these one-carbon precursors into nucleic acid purine as represented by urinary allantoin.

NUCLEIC ACID BIOSYNTHESIS

It seemed possible that vitamin B_{12} might be involved in nucleic acid synthesis at a site other than purine synthesis, particularly in view of the fact that nucleotides will replace vitamin B_{12} for *L. leichmannii* and certain other microorganisms, while the purine or pyrimidine bases themselves will not.[48,49] For this reason, we have also studied the effect of vitamin B_{12} deficiency on the incorporation of various labeled compounds into the ribose-nucleic acids (RNA) and desoxyribose-nucleic acids (DNA) of pig, chick, and rat liver. These nucleic acids were isolated by the procedure of Bendich *et al.*[50] (modified by addition of a preliminary treatment with amylase to eliminate glycogen).

Chicks: Vitamin B_{12}-deficient and vitamin B_{12}-normal chicks were given single intraperitoneal injections of C^{14}-formate, C^{14}-formaldehyde, α-C^{14}-glycine, β-C^{14}-serine, and methyl-C^{14}-methionine. The birds, as stated earlier, were sacrificed after 48 hours and both DNA and RNA were isolated from the livers and their radioactivity determined. The data from these experiments are summarized in Table XXIX. As can be seen from this table, absolutely no differences in labeling were found between the vitamin B_{12}-deficient and the

TABLE XXIX

Effect of Vitamin B_{12} on Nucleic Acid Synthesis in Chicks

Compound injected *40 μc/kg* body wt	B_{12} status	Radioactivity isolated nucleic acids *cpm/mg*	
		RNA	DNA
Glycine-2-C^{14}	+	460	328
	−	388	305
Serine-3-C^{14}	+	308	325
	−	330	330
Formate-C^{14}	+	680	605
	−	625	582
Formaldehyde-C^{14}	+	590	482
	−	470	505

TABLE XXX

Effect of Vitamin B_{12} on Nucleic Acid Synthesis in Pigs

Compound injected 40 μc/kg body wt	B_{12} status	Radioactivity isolated nucleic acids cpm/mg	
		RNA	DNA
Formate-C^{14}	+	360	316
	−	318	356
Serine-3-C^{14}	+	266	232
	−	296	226

TABLE XXXI

Effect of Vitamin B_{12} on Glucose-C^{14} Metabolism in Pigs

Compounds isolated	$-B_{12}$ cpm/mg	$+B_{12}$ cpm/mg
RNA	260	255
DNA	235	230
Glycogen	392	420
Protein	180	265

Glucose injected at 40 μc/kg body wt.

vitamin B_{12}-normal birds, although, as might be anticipated, the total radioactivity in the case of the formate and formaldehyde-injected animals was high as compared to the other precursors (glycine, serine, or methionine).

Baby Pigs: C^{14}-formate, α-C^{14}-glycine, β-C^{14}-serine, and, in this case, uniformly labeled C^{14}-glucose were given by intraperitoneal injection to vitamin B_{12}-deficient and vitamin B_{12}-normal baby pigs and the animals sacrificed after four hours. Again both RNA and DNA were isolated from the livers and their radioactivity determined. The nucleic acid data from these pigs are given in Tables XXX and XXXI. As in the case of the chick, here again with the baby pig no effect of vitamin B_{12} deficiency was found on labeling of the nucleic acids. Thus, it appears that vitamin B_{12} is *not* directly involved in the incorporation of glycine, serine, methionine, formate, formaldehyde, or glucose into nucleic acids.[51] This would appear to rule out a role of vitamin B_{12} not only in purine biosynthesis in the animal but also elsewhere in nucleic acid synthesis. The glucose data would also appear to rule out a role of vitamin B_{12} in the sequence, glucose → ribose → desoxyribose.

Rats: β-C^{14}-serine was used as nucleic acid precursor in studies with rats made vitamin B_{12}-deficient on the soy-lactose ration. The data given in Table XXXII are seen to be in complete agreement with those from the chick and the baby pig.

TABLE XXXII

Effect of Vitamin B_{12} on Serine-3-C^{14} Metabolism in Rats

Compounds isolated	$-B_{12}$ cpm/mg	$+B_{12}$ cpm/mg
RNA and DNA	242	230
Glycogen	106	328
Protein	162	240

Serine injected at 40 μc/kg body wt.

PRELIMINARY STUDIES ON PROTEIN AND CARBOHYDRATE

In the case of the baby pig given C^{14}-glucose and of the rat given β-C^{14}-serine, we have isolated, in addition to the nucleic acids, glycogen and protein in the hope of spotting some more general functions of vitamin B_{12} than had yet been found. The data for these studies are given in Tables XXXI and XXXII and, while preliminary, they do appear to be most interesting. It is planned to concentrate further efforts in this area.

Our earlier report that cortisone enhanced the deficiency of vitamin B_{12} in baby pigs fed the deficient diet[52] and the recent reports on lower enzyme[23,53,54] and serum protein[55] levels in vitamin B_{12} deficiency would all appear to support the above postulation of a role of vitamin B_{12} in protein synthesis.[56]

SUMMARY

It appears that vitamin B_{12} is not directly involved in transmethylation in the animal body nor in the biosynthesis of liver ribose or desoxyribose nucleic acids. On the other hand, vitamin B_{12} is definitely required for methyl group synthesis from various one-carbon precursors including serine, glycine, formate, and formaldehyde, and its role in one-carbon metabolism appears to influence the incorporation of certain one-carbon compounds into the uric acid excreted by the chick. Preliminary evidence indicates the involvement of vitamin B_{12} in protein synthesis.

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Vitamin E (Tocopherol) in Human Tissues from Birth to Old Age*

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THERE IS a rather extensive literature dealing with blood levels of tocopherols in man. Plasma tocopherols tend to be reduced in diseases such as celiac disease, fibrocystic disease of the pancreas, sprue, obstructive jaundice and diarrhea associated with achlorhydria, which are characterized by impairment of fat absorption.[1-4] On the other hand, they tend to be elevated in diseases associated with increased blood lipids but are not significantly influenced by other metabolic diseases or by infectious diseases.[1] Average plasma tocopherol values reported for newborn infants range from 0.23 to 0.43 mg[5-8] and those for young and adolescent children average about 1.0 mg/100 ml,[8,9] as compared to a range of about 1.0 to 1.2 mg/100 ml for healthy adults. Relatively little attention has been given to the distribution and concentration of tocopherols in other tissues and organs in man, and the extent to which these may be influenced by disorders of fat absorption or by illnesses of other types. This is due largely to the fact that methods for the chemical measurement of tocopherols in tissues are decidedly laborious as compared to the more simple and rapid micromethod for plasma tocopherols.

Abderhalden,[10,11] who presented the first data on tocopherols in human tissues and organs, employed a fluorometric method of analysis to material obtained from four fetuses, three newborn infants and ten adults. He noted relatively low concentrations of tocopherols, in terms of mg/100 g fresh tissue, in most of the tissues and organs examined; notable exceptions were the adipose tissue and, especially, the adrenal gland. Somewhat higher values were subsequently reported by Quaife and Dju[12] for tissues and organs from two cases of accidental death in adults, and by Dju *et al.*[13] for material from 28 fetuses and 23 premature and full-term infants. These investigators employed a different method; namely, that of Quaife and Harris,[14] based on a colorimetric reaction applied to molecular distillates of lipid extracts of tissues, which presumably gives a more accurate evaluation of the tocopherols present.

As a continuation of previous observations of tocopherols in fetal and infant tissues,[13] this report deals with the tocopherol status of man from early infancy to advanced old age. A preliminary summary of data from both studies was presented at a previous symposium of the National Vitamin Foundation.[15] Certain of the data have since been corrected to make allowance for tocopherol loss during deep-freeze storage, on the basis of findings presented elsewhere,[16] and the individuals representing the source of tissues and organs analyzed have been grouped somewhat differently in order to better compare the tocopherol status of those

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in which death was accidental with those in which death followed varied types of disease processes.

MATERIALS AND METHODS

Materials

Tocopherol analyses were carried out on samples of tissues and organs from 70 individuals. These included nine infants (age one to eight months), 14 children (age one to ten years), 12 adolescents (age 12 to 18 years) and 35 adults (age 23 to 93 years). There were 14 instances of accidental deaths, including seven children, five adolescents and two adults. In five other adults death was due at least secondarily to chronic alcoholism; in 16 others cardiovascular disease such as coronary or generalized arteriosclerosis, coronary occlusion or cerebral accident was the primary cause of death. Since neither the clinical history nor necropsy findings revealed evidence of other disease processes of major consequence, these 16 individuals may be considered reasonably representative of average normal individuals at their age periods (34 to 93 years). Also included for purposes of comparison are data on 29 premature and full-term infants succumbing at birth or during the first week of life, representing an extension of data on 23 premature and full-term infants previously recorded.[13] The latter study and the present report are based upon analyses of more than 1,000 specimens of human tissues and organs.

Samples of adipose tissue (subcutaneous and retroperitoneal, combined), liver, skeletal muscle (pectoralis major and psoas major, combined) and heart muscle were taken routinely when procurable. These four tissues were selected because they are usually obtainable for chemical analysis at necropsy in desired amounts (5 to 10 g) and because, combined they represent slightly more than one-half the total body mass. Furthermore, since the tocopherol content of most visceral organs is of much the same order as that of skeletal muscle, heart, and liver,[9–12] it was believed that the data obtained regarding these four tissues should provide a reasonably good evaluation of the tocopherol status of the body as a whole. However, to extend existing information on other tissues, analyses were carried out on a variety of other visceral organs obtained chiefly from the necropsies which provided the other tissues mentioned.

Methods

The fresh tissues were trimmed of extraneous fat, tied in cellophane or plastic bags, and placed in deep-freeze storage at −20° C until analyzed. Tissues shipped from outside sources were treated likewise, and kept frozen with dry-ice while in transit. Total tocopherols were determined by the macrochemical procedure of Quaife and Harris[14] for tocopherols in food products, as modified by Quaife and Dju[12] for tocopherols in fresh animal tissues. In brief, the method comprises homogenization of tissue after freezing in liquid nitrogen, extraction with hot ethanol for 24 hours, addition of water to the alcoholic phase, concentration of lipids and tocopherols in a Skellysolve B phase, molecular distillation to concentrate and separate tocopherols from lipids, and hydrogenation to remove interference due to carotenoids and vitamin A. Total tocopherols were measured by the ferric chloride-dipyridyl reaction of Emmerie and Engel on an aliquot of the distillate. The diazo method of Weisler *et al.*[17] was used to measure gamma- and delta-tocopherols, combined, on another aliquot of the distillate. The lipid content of the tissue was obtained by calculating the weight of the extract obtained from the Skellysolve B fraction. Values were obtained and recorded for lipid content, for total tocopherols as mg/100 g fresh tissue and as mg/g of fat, and for combined γ- and δ-tocopherols* as mg/100 g fresh tissue.

* Since these studies were carried out, three other tocopherols (epsilon, zeta and eta) have been isolated from vegetable sources by paper chromatography and identified chemically.[18] Since only one of these (η, or 7-methyltocol) reacts with the diazo reagent employed in our studies for the measurement of γ- and δ-tocopherols, it is apparent that our values for the latter two compounds actually represent values for combined γ-, δ- and η-tocopherols, if it be shown eventually that the eta form normally occurs in human tissues. It should also be mentioned that the color reaction on which identification of these tocopherols is based lacks a certain degree of sensitivity, such that zero values recorded (Table II) do not deny the presence of traces or small amounts in the tissues studied.

Tissues obtained locally were usually analyzed within two to three weeks after procural; certain of those obtained from outside sources, because of delays in accumulating and shipping the specimens, were subjected to storage for longer periods, but rarely more than ten weeks. The tocopherol values for adipose tissue and liver have been adjusted for loss during deep-freeze storage, on the basis of data reported elsewhere;[16] the tocopherol values for all other tissues presented in this report are recorded as obtained values, which we have reason to believe are within 90 to 100 per cent of what the values would have been prior to storage.

OBSERVATIONS AND RESULTS

To facilitate presentation of results, 58 of the 70 cases serving as sources of the tissues studied have been arbitrarily divided into ten groups on the basis of age and primary cause of death or major clinical findings; the 12 others constitute a rather miscellaneous group of adults which will be discussed separately. Table I presents average values for lipid and for tocopherol content of four tissues in these ten groups (B to K) and, for comparison, comparable data on 29 newborn infants (premature and term) less than one week old (group A). Table II gives data on each of the 14 individuals in the four groups (D,E,G,H) representing cases of accidental death, and on each of the 12 adult individuals in the miscellaneous group not incorporated in Table I.

The values recorded in Table II serve to illustrate the rather wide range in values for lipids and tocopherols in specific tissues from any one group, and the difficulty in determining what may be considered normal for any given age range. Considering these inherent variations and the limited number of individuals in most groups, it is apparent that the average values presented in the summary data of Table I and Figure 1 should be interpreted largely in terms of indicated *trends* in tocopherol status in relation to age and to body states.

TABLE I

Lipid and Tocopherol Content of Adipose Tissue, Liver, Skeletal Muscle and Heart Muscle from Individuals Grouped According to Age and Cause of Death, Expressed as Averages for Each Group*

Group	No. Cases	Adipose Tissue		Liver		Skeletal Muscle		Heart	
		Lipid %	Tocopherol *mg/100 g*	Lipid %	Tocopherol *mg/100 g*	Lipid %	Tocopherol *mg/100 g*	Lipid %	Tocopherol *mg/100 g*
A Infants (0–7 days; premature and term)	29	35.5^{24}	2.6	3.6^{29}	1.1	2.2^{29}	0.6	2.2^{23}	0.7
B Infants (1–8 mo)	9	42.9^{9}	1.7	5.5^{9}	0.9	2.5^{9}	0.5	3.2^{3}	0.3
C Children (1–5 yr; varied illnesses)	7	50.2^{5}	4.8	3.9^{6}	0.6	1.9^{5}	0.4	3.2^{3}	0.8
D Children ($1^1/_2$–4 yr; accidental death)	3	57.3^{3}	7.5	4.2^{3}	2.0	2.3^{3}	0.8	2.8^{3}	1.2
E Children (6–10 yr; accidental death)	4	47.0^{4}	7.7	4.0^{4}	1.6	2.8^{4}	1.3	3.7^{4}	1.5
F Adolescents (12–17 yr; varied illnesses)	7	60.5^{7}	6.9	5.0^{6}	1.1	4.9^{7}	1.0	2.4^{1}	0.9
G Adolescents (13–18 yr; accidental death)	5	45.5^{5}	16.1	4.2^{5}	2.2	2.8^{5}	0.9	3.3^{4}	1.1
H Adults (23–31 yr; accidental death)	2	68.7^{2}	24.5	5.4^{2}	2.5	2.6^{2}	1.2	2.6^{2}	0.8
I Adults (32–52 yr; alcoholics)	5	69.0^{5}	7.5	18.6^{5}	3.4	3.9^{5}	1.1	3.7^{4}	1.4
J Adults (34–50 yr; vascular disorders)	6	72.0^{6}	9.5	4.9^{6}	2.1	3.3^{6}	1.3	2.6^{5}	1.1
K Old Age (61–93 yr; vascular disorders)	10	66.9^{10}	7.3	4.4^{9}	1.1	7.2^{6}	0.9	3.4^{6}	1.1

* Superscript numbers indicate the number of individuals in each group represented in the tissue samples analyzed.

TABLE II

Lipid and Tocopherol Content of Adipose Tissue, Liver, Muscle and Heart in 14 Cases of Accidental Death and in 12 Cases of Death Due to Miscellaneous Causes

Group	Sex	Age	Cause of Death or Major Clinical and Necropsy Findings	Adipose tissue		Liver		Muscle		Heart	
				Lipid %	Tocopherol *mg/100 g*	Lipid %	Tocopherol *mg/100 g*	Lipid %	Tocopherol *mg/100 g*	Lipid %	Tocopherol *mg/100 g*
	M	18 mo	Drowned in bath tub	58.6	9.8	4.4	0.9	1.2	0.4	2.5	1.0
D	M	2½ yr	Ingestion of detergent	56.7	4.1	4.9	1.8	2.7	0.7	2.7	1.5
	M	4 yr	Auto accident	56.6	8.6	3.4	3.2	2.9	1.2	3.1	1.2
			Av	57.3	7.5	4.2	2.0	2.3	0.8	2.8	1.2
	M	6 yr	Auto accident	57.1	5.3	4.0	1.7	2.3	0.8	4.3	1.8
E	M	7 yr	Auto accident	41.0	9.5	4.4	1.8	3.1	1.7	2.5	2.4
	M	9 yr	Drowned in lake	43.0	5.6	3.3	1.9	2.0	1.7	2.5	0.9
	F	10 yr	Auto accident	46.7	10.3	4.2	1.0	4.3	1.0	5.6	1.0
			Av	47.0	7.7	4.0	1.6	2.8	1.3	3.7	1.5
	M	13 yr	Head crushed by lift	41.9	1.6	3.0	1.4	2.4	0.9	2.5	0.8
	M	14 yr	Fracture of skull	33.0	5.2	4.0	1.0	3.6	0.4	4.1	1.6
G	M	17 yr	Accident	53.1	21.4	5.8	3.1	2.7	1.4	—	—
	M	17 yr	Gunshot wound	65.7	33.6	3.7	1.8	1.4	0.8	2.3	1.4
	M	18 yr	Auto accident	33.6	18.2	4.6	3.8	4.0	1.0	4.3	0.5
			Av	45.5	16.1	4.2	2.2	2.8	0.9	3.3	1.1
H	M	23 yr	Auto accident	61.3	31.7	4.1	1.5	1.9	1.4	2.9	1.0
	M	31 yr	Auto accident	76.0	17.3	6.7	3.5	3.2	1.1	2.3	0.7
			Av	68.7	24.5	5.4	2.5	2.6	1.2	2.6	0.8
	M	26 yr	Visceral congestion	61.6	3.5	4.0	0.7	1.5	0.7	2.3	0.5
	F	32 yr	Visceral congestion	83.5	9.6	3.5	1.2	2.2	0.8	3.5	1.0
	F	39 yr	Hepatitis	67.8	19.2	4.9	3.5	6.3	3.1	3.2	1.3
	M	46 yr	Malignant hypertension	73.9	22.3	4.3	1.7	3.0	1.5	3.5	0.9
	M	87 yr	Lobar pneumonia; shock	70.3	13.1	6.9	1.9	2.2	0.9	3.1	1.1
L	M	21 yr	Proto. musc. dystr.; pneum.	82.3	8.6	8.0	3.3	32.7	5.5	9.9	1.9
	M	46 yr	Amyotrophic lateral sclerosis	74.9	30.0	4.4	1.0	4.9	4.2	—	—
	F	63 yr	Myasthenia gravis	66.4	50.8	4.4	0.8	4.8	1.2	—	—
	F	28 yr	Brain tumor (8 mo preg.)	56.8	10.2	3.0	1.8	3.0	0.7	—	—
	F	34 yr	Brain tumor	82.5	5.2	8.8	1.2	5.3	1.8	3.3	1.0
	M	59 yr	Carcinoma of pancreas	64.6	1.1	4.3	0.7	2.3	0.6	—	—
	F	86 yr	Metastatic carcinoma	48.3	5.7	3.1	0.9	7.5	6.6	3.7	0.5

Tissue Tocopherols in Relation to Accidental Death

Only 14 individuals, or 20 per cent of the total number of subjects from whom tissues were obtained, represented instances of accidental death (Groups D,E,G,H, Tables I and II). None of these presented evidence of active disease processes, and therefore may be considered to have been reasonably normal, healthy individuals for their age. Although several chronic alcoholics also died accidentally as a result of falls or foul play, it seems more appropriate to include them in a separate group (Group I, Table I). Thirteen of the 14 individuals comprising the accidental death group were males.

The lipid content of the four tissues recorded (Table II) varied within moderate limits and showed no particular trends in relation to age. The total-tocopherol values, whether expressed as mg/100 g fresh tissue (mg %) or as mg/g of fat, varied over a somewhat wider range; however, only in the case of adipose tissue was there a significant difference in relation to age, in that values tended to be appreciably higher in late adolescents and young adults (17 to 31 years) than in younger individuals. Although adipose tissue levels of this degree (17 mg/100 g, or greater) were not often observed in adults dying of other causes, comparable levels have been reported[11] for two other cases of accidental death in adults. The data in Table II also indicate that high or low levels of tocopherols in any one tissue bore no particular relationship to levels in other tissues from the same individual. Although some of this individual

tissue variability can be attributed to errors in sampling, much of it appears to be inherent in the tissues themselves, *i.e.* in the individual serving as the source material.

γ- and δ-tocopherols (and presumably η-tocopherol also) represented a decidedly variable proportion of the total tocopherols. In certain instances they were not measurable, and rarely did they exceed 25 per cent of the total tocopherols. They tended to comprise proportionately more of the total tocopherols of adipose tissue than of skeletal or cardiac muscle, with the liver assuming a somewhat intermediate position.

Tissue Tocopherols in Relation to Age and Cause of Death

In Table I (Groups B to K) are presented data on the average lipid and tocopherol values for the four tissues most commonly obtained (adipose tissue, liver, skeletal muscle and heart) from 58 individuals ranging in age from one month to 93 years of age, grouped according to age and cause of death. For comparison with the tocopherol status of the same tissues at birth, there are included average values for 29 premature and full-term infants, less than one week of age (Group A).

(a) *Lipids:* The lipid values for liver, skeletal muscle and heart were of much the same order in all groups, except for rather high liver lipids in the chronic alcoholic subjects (I) and moderately high muscle lipids in the old age group (K); the somewhat high value for muscle lipids in the adolescent group (F) is due to an exceptionally high level (12.18 per cent) in one individual, and appears to have no real significance. For adipose tissue the lipid values were more variable, and tended to be lower in infancy and higher in adulthood than at intermediate age periods.

(b) *Total Tocopherols, as mg/100g fresh tissue:* When expressed as mg/100 g fresh tissue, tocopherol values for the four tissues recorded (Table I) were somewhat lower in infants succumbing to congenital defects and other disorders at one to eight months of age (B) than in newborn infants (A), whose death was usually ascribed to atelectasis. In children succumbing to varied illnesses at one to five years of age (C), tocopherols in liver and skeletal muscle were lower than in infants at birth or at one to eight months of age; those in adipose tissue were generally low, although a rather high value which was obtained in one case (11.8 mg/100 g) considerably increased the average for the group (from 3.0 to 4.8 mg/100 g). On the other hand, in children one and one-half to four and six to ten years of age (D,E), whose deaths were due to accidental causes, tissue tocopherols were appreciably higher than in those of the groups previously considered (A,B,C). Likewise, tissue tocopherols in adolescents succumbing to varied illnesses (F) were appreciably lower in adipose tissue and liver than in adolescents of comparable age whose death was accidental (G). When average tissue tocopherol values for all 12 instances of accidental death in the age range of 1-17 years (D,E,G) were compared to the 14 instances where death was due to varied illnesses at comparable ages (C,F), the respective ratios were 11.2:6.0 for adipose tissue, 2.0:0.9 for liver, 1.0:0.7 for skeletal muscle, and 1.3:0.8 for heart, thus indicating a definite depression of tissue tocopherols as a result of acute and chronic illnesses.

In the group of five chronic alcoholic subjects (I, Table I) death in at least three instances was by accident or foul play. However, these alcoholics differ from the two cases of accidental death (H) in two major respects; much lower tocopherol levels in adipose tissue and a rather high lipid content of the livers, which grossly showed evidence of fatty infiltration. Although liver tocopherols appear to be somewhat higher than in all other groups when expressed as mg/100 g, since the increase in liver fat means a related increase in tocopherols per unit of liver tissue, they are relatively low when calculated in terms of mg/g of fat.

In the younger adults representing vascular disorders (J) there was one case of rheumatic heart disease, one case of brain hemorrhage, and two each of coronary and of generalized arteriosclerosis. Except for diminished tocopherols in adipose tissue, the findings do not differ significantly from those in cases of accidental death (H).

The group of ten older adults (K) represent

eight cases of generalized arteriosclerosis and two cases of coronary occlusion. These subjects likewise show diminished tocopherols in adipose tissue, and indications of fatty infiltration of skeletal muscle associated with decreased concentration of tocopherols. Tocopherol values for liver are somewhat low, those for heart muscle reasonably normal.

The data obtained from these groups of adults (32–93 yrs.; I,J,K), although lacking comparative data from tissues of vigorous and presumably healthy individuals of comparable age, do suggest that tissue tocopherols of adipose tissue tend to reach a maximum in healthy adolescents and young adults, and tend to diminish appreciably after the third decade of life. The data show also that the tocopherols of liver and skeletal muscle are influenced by fatty infiltration as the result of chronic alcoholism or advanced age, while those of cardiac muscle are not influenced significantly.

(c) *Total Tocopherols, as mg/g of Fat:* Tocopherols are natural components of cell lipids. It has been our experience that tocopherols when expressed as mg per 100 g tissue present a similar, but often not quite as adequate a picture of the tocopherol status of tissues as when expressed in terms of mg/g of fat. This is related to the fact that variabilities in the lipid content of certain organs and tissues tend to be associated with greater variations in the amounts of tocopherol per unit weight of tissue than in the concentration of tocopherol in the cell lipid itself.

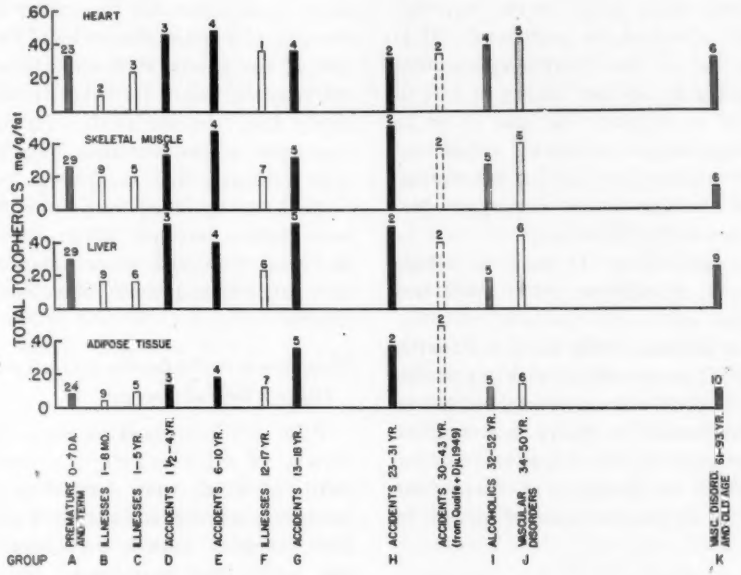

Fig. 1. Graphic representation of average tocopherol values (expressed as mg tocopherol/g of fat) for adipose tissue, liver, skeletal muscle and heart, in groups A to K of Table II. Figures above each column indicate the number of subjects in each group serving as a source of the tissue samples from which the average tocopherol values were obtained.

Figure 1, which records graphically the tissue tocopherol levels expressed as mg/g of fat for the same groups of subjects listed in Table I, suggests that this method of expressing tocopherols permits a more valid comparison between adipose tissue, fatty livers of chronic alcoholics, and other organs studied.

In close accord with interpretations made on

the basis of the data recorded in Table I, those of Figure 1 clearly indicate that:

(a) Illness in early infancy (B) was associated with lower tissue tocopherol levels than observed in premature and term infants (A).

(b) Illness during the age periods 1 to 5 and 12 to 18 (C,F) was characterized by tocopherol levels considerably lower than in instances of accidental death at these ages (D,G) or at 6 to 10 years of age (E).

(c) Relatively high tissue levels, approximating those observed in adulthood (H,J) were also typical of the groups representing accidental deaths in the age ranges of $1^1/_2$ to 4 years (D), 6 to 10 years (E) and 13 to 18 years (G), suggesting a relatively rapid and approximately maximal acquisition and storage of tocopherols by these tissues and organs during infancy and early childhood.

(d) Chronic alcoholism (I) tends to reduce tocopherol levels in adipose tissue, liver and skeletal muscle.

(e) Vascular diseases in the third and fourth decades of life (J) are associated with depression of tocopherol levels in adipose tissue, but not in heart, skeletal muscle or liver; however, at more advanced ages (K) there is a tendency for tocopherol levels to diminish in these four tissues and to approach those observed in early infancy.

Miscellaneous Group of Adults

The data presented (Table II) for the 12 adults in the age range of 26 to 86 years, considered separately because they did not logically fit criteria of the groups previously considered (Table I), serve to indicate again the difficulty in correlating cause of death or nature of prolonged illness with the lipid or the tocopherol status of any particular organ or tissue. However, a few of the findings appear to have some significance.

In the subject succumbing to progressive muscular dystrophy, on whom more detailed data are presented elsewhere,[19] the rather high lipid and tocopherol content of skeletal muscle (based on samples from eight different muscles) and the rather low tocopherol content of adipose tissue may reflect widespread fatty infiltration of the dystrophic musculature associated with a general dilution of tocopherols secondary to the greatly increased body fat. There was no history of vitamin E medication, during the last few years of life. The unusually high tocopherol values for adipose tissue in the victims of amyotrophic lateral sclerosis and myasthenia gravis may possibly be correlated with evidence from the patients' history that tocopherol medication had been given at intervals during the last few months of life. There is no explanation for the rather high level of tocopherol in skeletal muscles of the oldest subject of the group, with metastatic carcinoma, whereas the values for other tissues were relatively low. In the next older subject, with carcinoma of the pancreas, data not recorded here indicated that tocopherol levels in both normal and involved portions of the pancreas were within normal limits for this organ. Analyses of several other pancreata with extensive carcinomatous involvement gave similar results.

Tocopherols in Endocrine Glands and Other Visceral Organs

Table III records data from analyses of a variety of other organs, approximately two-thirds of which were derived from the same necropsies serving as a source of adipose tissue, liver, skeletal muscle and heart previously discussed. For comparison, there are also included data on adipose tissue from the 18 subjects from whom the adrenals were obtained.

Compared to the levels in liver, from cases of accidental death (Table II), tocopherols (mg/100 g) were of about the same order in pancreas but somewhat lower in lung, kidney, spleen, uterus and ovary; but on the basis of mg/g of fat, tocopherols were higher and lipids lower in uterus and ovary than in the other tissues mentioned. Tocopherols were considerably higher in testis and pituitary regardless of the criterion used. On the basis of mg/g of fat, tocopherols were higher in the pituitary than in any other organ or tissue examined, while the testis ranked second. The adrenal, on the other hand, when compared to adipose tissue from the same subjects, had about one-third as much lipid, somewhat more tocopherol per unit weight, and about five

TABLE III

Tocopherols in Various Tissues

Tissue	Age of subjects yr	Number	Lipids % (range)	Total Tocopherols	
				mg % (range)	mg/g fat (range)
Lung	61–91	4	2.0 (1.5–2.7)	0.4 (0.3–0.5)	0.2 (0.1–0.3)
Kidney	17–90	7	3.0 (2.1–3.7)	0.7 (0.2–1.0)	0.3 (0.05–0.4)
Spleen	12–90	10	2.4 (1.4–3.3)	0.8 (0.2–1.3)	0.3 (0.1–0.5)
Pancreas	23–91	19	8.0 (2.9–20.4)	1.8 (0.8–4.0)	0.3 (0.04–0.7)
Uterus	28–45	5	1.4 (0.9–2.2)	0.9 (0.3–1.4)	0.7 (0.2–1.5)
Ovary	32–50	5	1.6 (1.3–2.3)	1.1 (0.4–2.1)	0.6 (0.3–1.9)
Testis	21–50	11	3.8 (2.0–10)	4.0 (1.3–12)	1.0 (0.6–1.8)
Pituitary	26–50	22	3.6 (1.6–10)	4.0 (0.5–12)	1.2 (0.2–3.8)
Adrenal	21–77	18	26 (5.1–69)	13.2 (2.9–24)	0.7 (0.05–3.5)
Adipose tissue	21–77	18	71 (55–82)	10.4 (3.6–25)	0.15 (0.05–0.4)
Blood	Estimates		0.5–0.6	1.0–1.2	2.0 (approx.)

times as much tocopherol per gram of lipid; thus, it ranked highest of all organs and tissues on the basis of tocopherols per unit weight (mg/100 g). The data were insufficient to indicate whether there were trends in tocopherol content which might be related to age, sex, or cause of death.

DISCUSSION

The data presented here, combined with those of an earlier report,[13] provide a spectrum of tocopherols in human tissues from early fetal life to advanced old age, and generally confirm other reported observations[11,12] based on a more limited number of subjects. In fact, we now have a much more complete picture of the distribution and relative concentration of vitamin E in human tissues than for any of the other vitamins. Although the information gained has as yet provided no significant insight into the metabolic functions of vitamin E in the animal organism, the data have brought out several interesting points which warrant brief comment.

During the first decade of postnatal life, tocopherols appear to normally increase almost two-fold in liver, skeletal muscle and heart, and about three-fold in adipose tissue, such that the values attained approach the maximum for normal adults (except for adipose tissue on a mg/100 g basis). Tocopherols in blood show a similar (two- to three-fold) increase during early life and attain adult levels (1.0 to 1.2 mg/100 g) of much the same order as observed in the above tissues, and those found in lung, kidney spleen, pancreas, uterus and ovary. In adipose tissue tocopherols appear to increase more rapidly and progressively, attaining values in young adults about eight times those at birth. The latter undoubtedly represents increase in both lipid content and tocopherol deposition in adipose tissue. However, the latter tissue cannot be regarded as a storage depot for the vitamin in the same sense that the liver functions for vitamin A, for when tocopherols of adipose tissue are calculated as mg/g of fat their actual concentration in the cell lipids is much less than for any other tissue of the body (Fig. 1). On the other hand, assuming blood tocopherol levels of 1.2 mg/100 ml and blood lipids of 600 mg/100 ml for a healthy adult, blood would contain 2 mg tocopherols/g of fat and represent the tissue with the greatest lipid concentration of tocopherol.

The fact that tocopherol concentration in blood lipids exceeds that in the lipids of other tissues and organs, and that the lipoproteins of blood serve as "carriers" of tocopherol,[20] may have some bearing upon the manner by which tocopherols are transferred from blood to tissue cells and also upon limitations in the extent to which this can occur. It is generally believed that tocopherols are incorporated in cells as a natural component of lipids acquired by the cell during normal metabolic processes, and

that they are lost from the cell in a reverse manner.

It may be noted that there was no consistent or significant trend toward lower lipid levels in tissues from subjects succumbing to varied illnesses, when compared to those whose death was accidental (Table I). This suggests that the lower tissue tocopherol levels of those with varied illnesses may be more a reflection of retarded deposition than one of actual loss of cell tocopherols. Unfortunately there are no data on plasma tocopherols in these subjects prior to death. However, it is reported that plasma tocopherol levels are not depressed by metabolic and infectious diseases and tend to be in the normal range or even elevated in many diseases involving the kidney, heart or liver,[21–23] especially if there is an associated hypercholesteremia.[1] Plasma levels are essentially normal in middle-aged and old individuals on a diet adequate in vitamin E.[24]

It is now recognized that critically low blood tocopherol levels, associated with marked susceptibility of red cells to hemolysis, occur commonly in infants with biliary atresia, steatorrhea, pancreatic fibrosis or celiac disease[3,4] and also as a result of experimental depletion of vitamin E in adult humans.[25] It is noteworthy that in groups B, C and F (Table I) there were, respectively, one case of jaundice, three cases of jaundice, and one case of celiac disease; however, the tocopherol levels in the tissues examined showed no pattern that would distinguish them from infants or young children succumbing to a variety of other diseases, such as renal disorders, congenital heart disease, respiratory diseases and brain tumors. This may mean that in these subjects impairment of fat absorption was not severe enough to seriously limit tocopherol absorption, or became operative after considerable tissue-tocopherol deposition had occurred.

The pattern of wide distribution of tocopherols in human tissues, in amounts which are somewhat variable but generally within a limited range, is in accord with what is known regarding tocopherols in tissues of lower animals and is compatible with the generally accepted hypothesis that tocopherols (especially α-tocopherol) function as important intracellular antioxidants, necessary perhaps for the stabilization of cell lipids and their oxidation products. On the other hand, the relatively high concentration of tocopherols in the pituitary in particular, and also in the testis and adrenal, for which no adequate explanation can yet be given, suggests the possibility of other functions. This might connote either a particularly high requirement for tocopherols for the normal metabolic activities of these organs, or a low rate of utilization at such a degree that accumulation or true storage of tocopherols occurs. The recent studies of Rosenkrantz[26,27] indicating that the hydroquinone and quinone of α-tocopherol play an important role in the synthesis of adrenal steroids in the rabbit, which also has a high concentration of tocopherol in its adrenal gland, would support the first alternative. The fact that tocopherols are present at higher concentrations in the pituitary than in the adrenal of both rat[28] and man suggests, at least, that tocopherols are important in metabolic activities of secretary cells other than those related to steroid production. It is also of some interest that the average values for total tocopherols of human pituitary, adrenal and testis, expressed as mg/g of fat (Table III), are essentially identical to those recorded for the normal rat by Quaife *et al.*[28] Furthermore, there are other similarities between the distribution of tocopherols in tissues of the rat, whether determined by chemical[28] or by biologic assay,[29] and those observed in man. At present, there exists at least the hope that the data with respect to tissue-tocopherol relationships presented here, combined with information now at hand and knowledge yet to be revealed, may provide a more adequate understanding of the true functions of vitamin E in man and the lower animals.

SUMMARY AND CONCLUSIONS

Samples of adipose tissue, liver, skeletal muscle and heart were obtained routinely (whenever available) at necropsy of 70 individuals, and analyzed chemically for total tocopherols. These included 9 infants (1–8 mo) 14 children (1–10 yr), 12 adolescents (12–18 yr)

and 35 adults (23–93 yr). Fourteen of these represented accidental deaths and included seven children, five adolescents and two adults. In five adults death was due at least secondarily to chronic alcoholism; in 16 others cardiovascular diseases (coronary occlusion, coronary or generalized arteriosclerosis, or cerebral thrombosis) were the primary causes of death. Comparable data from 29 premature and full-term infants are presented.

Tocopherols calculated as mg/g of fat seemed to give a more adequate evaluation of tocopherol status of the individual than when expressed as mg/100 g tissue. By the former criterion, the tissue levels never exceeded the accepted normal level of blood tocopherols. Alpha tocopherol predominated; γ and δ tocopherols, combined, rarely comprised more than 25 per cent of the total tocopherols, and often were not present in measurable amounts.

In liver, skeletal muscle, and heart from normal subjects (accidental death) total tocopherols appeared to reach maximal values during late childhood and adolescence, whereas those of adipose tissue increased more progressively through adolescence and early adulthood.

In infants, children and adolescents, death due to congenital defects and illnesses of varied types was associated with tissue tocopherol levels significantly lower than in normal subjects of comparable age. In adults, chronic alcoholism tended to reduce tocopherols in adipose tissue, liver and skeletal muscle.

During the third and fourth decades of life there was a diminution of tocopherols in adipose tissue, whereas at more advanced ages tocopherols in all four tissues tended to diminish and to approach levels observed at birth. These phenomena appeared to be related more to the normal process of aging than to the vascular disorders which constituted the primary cause of death.

Tocopherol levels in lung, kidney, spleen and pancreas were of somewhat the same order as in liver, skeletal muscle and heart. Those in pituitary, testis and adrenal were considerably higher. In terms of mg/g of fat, tocopherols were higher in the pituitary than in any other tissue, with the testis ranking second, the adrenal third, and adipose tissue lowest of all tissues examined.

When expressed as mg/100 g tissue, tocopherols were highest in adrenals and adipose tissue and about one-third as high in pituitary and testis, which ranked several times higher than other tissues studied.

The above statements are based upon average values which, to a reasonable degree, compensate for rather wide variations in tocopherol levels observed, even in the same tissue or organ from different individuals otherwise comparable from the standpoint of age and cause of death. Data available indicate that the variable pattern in tocopherol content of human tissues is largely a reflection of varied dietary intake and absorption of tocopherols.

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Effect of Storage on Tissue Tocopherols*

By Mei Yu Dju, ph.d.,† Lloyd J. Filer, Jr., ph.d., m.d.,† and Karl E. Mason, ph.d.

In procedures involving chemical or biologic evaluation of tocopherols in tissues of man or lower animals there often are variable and unavoidable intervals of time between acquisition and analysis of tissue samples. In the case of human tissues there are also variable intervals between death and necropsy. There appear to be no recorded observations on the rate and extent of loss of tissue tocopherols under such conditions. The studies reported here, representing a phase of an extensive exploration of the tocopherol content of human tissues,[1,2] are concerned with the effects of postmortem storage at 4° C and at −20° C upon tocopherols in certain human tissues.

METHODS AND RESULTS

Tocopherols were determined by the macrochemical procedure of Quaife and Harris[4] as modified for fresh animal tissues by Quaife and Dju[5] and calculated as mg per cent (mg total tocopherols mg/ 100 g fresh tissue). Test experiments with pure tocopherols added to fresh liver, muscle and lard gave recovery values ranging from 92 to 101 per cent indicating the degree of accuracy of the procedure. The tissues employed and conditions of storage are given in the outline of individual experiments.

Storage at 4° C for Eight Days

Samples of five tissues of four premature infants were taken at necropsy, and again after storage of the corpse for two, four, or eight days in a refrigerator maintained at about 4° C (Table I). Tocopherol loss was greatest in adipose tissue, amounting to about 30 per cent after only four days as compared to losses of 32 and 22 per cent in liver after eight days. Tocopherols of skeletal muscle and brain decreased somewhat less, whereas those of lung tissue suffered but little change after eight-day storage.

TABLE I

Tocopherol Loss in Tissues of Infants During Storage at 4° C

Tissue	Storage days	Tocopherol, mg/100 g		Loss, %
		Fresh	Stored	
Muscle	2	0.74	0.68	8.1
Muscle	4	0.52	0.43	17.3
Adipose tissue	4	2.80	1.95	30.0
Muscle	8	0.45	0.38	15.5
Liver	8	1.38	0.93	32.2
Brain	8	0.15	0.13	13.3
Lung	8	0.72	0.69	4.1
Liver	8	1.51	1.18	21.8
Brain	8	0.36	0.29	19.4
Lower extremity		Skin left on	Skin removed	Difference, %
Muscle	8	0.58	0.52	10.3
Adipose tissue	8	1.71	1.23	28.1

The skin was removed from the lower extremity of a premature infant and the other extremity left intact. Both were stored for eight days under the same conditions. The tocopherol values for muscle and adipose

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* The data presented are based largely upon a thesis of Mei Yu Dju submitted to the Graduate Faculty of the University of Rochester in partial fulfillment of the requirements for the Ph.D. degree.

A preliminary report of these studies[3] was presented before the American Institute of Nutrition, at Cleveland, Ohio, in 1951.

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tissue from the skinned extremity were, respectively, 10 and 28 per cent less than for the same tissues from the other extremity (Table I), indicating that exposure to air is also a factor causing diminution of tissue tocopherols during storage.

Storage at −20° C for Eight Weeks

Samples of fresh tissues from a 28-year-old female, the victim of a brain tumor at the eighth month of pregnancy, were analyzed before and after eight weeks of deep-freeze storage at about −20° C. The tissues were tied in plastic bags from which excess air had been excluded. Tocopherol loss was again highest in adipose tissue, and appreciably less in liver, skeletal muscle, and kidney (Table II).

TABLE II

Tocopherols of Adult Human Tissues Analyzed at Time of Autopsy and Again after Eight Weeks Storage at −20° C

Tissue	Lipid, %	Total tocopherols, mg/100 g fresh tissue: At autopsy	Lipid, %	After storage for 8 weeks	Loss of tocopherols, %
Para-renal fat	50.46	8.59	50.08	5.19	39.5
Mesenteric fat	63.03	11.89	62.30	7.90	33.5
Liver	3.20	1.81	3.67	1.44	20.4
Muscle	2.96	0.71	2.10	0.58	18.3
Kidney	3.20	0.51	3.43	0.44	13.7

Storage at −20° C for Periods up to 109 Weeks

Large samples of adipose tissue and liver from a 62-year-old male, whose death resulted from coronary occlusion, were frozen in liquid nitrogen and ground in the usual manner. The frozen ground material was divided into 48 aliquots for each tissue, such that four aliquots could be used for the initial analyses and for each of 11 subsequent analyses after storage intervals of 1, 2, 4, 7, 9, 11, 19, 24, 34, 52, and 109 weeks. The aliquots were placed in extraction thimbles enclosed in plastic bags from which excess air was excluded prior to deep-freeze storage. At each interval, the lipid extracts obtained were also characterized as to iodine value and peroxide value both prior to and following concentration of tocopherols by molecular distillation.

The data on tocopherol levels, expressed as mg per cent and as per cent of initial values, are summarized in Figures 1 and 2. The ordinates for the two tissues recorded in Figure 1 serve to indicate that, per unit weight of fresh tissue, tocopherol values for adipose tissue were approximately ten times those for liver; however, when expressed in terms of mg/g of fat, tocopherol values for adipose tissue were only about one-third those of liver. There may also be noted an unexplained increase in tocopherol values for adipose tissue during the first week, and for liver during the first two weeks of storage.*

It is apparent from the graphs that the tocopherol content of adipose tissue was significantly lowered by the 4th week of storage, to about 79 per cent of the original value; subsequently there was a progressive decline to 66, 55, 52, 55 and 50 per cent at 9, 11, 19, 24, and 34 weeks, and to 33 and 24 per cent after 52 and 109 weeks, respectively. The tocopherol of liver was reduced more gradually, especially during the first 24 weeks (Fig. 1 and 2), such that at 4, 9, 11, 24, 34, 52, and 109 weeks, respectively, the values were 93, 78, 74, 60, 54, 37, and 34 per cent of those prior to storage.

Tocopherols expressed as mg/g of fat showed essentially the same relative decline, which may be correlated with the fact that the extractable fat of both tissues was remarkably constant at all phases of storage (mean value of 82.45 ± 1.43 per cent for adipose tissue; 3.76 ± 0.25 per cent for liver) during the 2-year period. The lipid extract of tissue samples analyzed at each interval up to 52 weeks, determined both before and after molecular distillation, gave mean iodine values of 69.1

* Since subsequent analyses of other adipose tissue and of liver treated in like manner failed to show any increase of tocopherol values during the early weeks of storage, the increased levels recorded are of questionable significance; they may be related to differences inherent in the aliquots employed or to limitations of the analytical procedure employed, or to both.

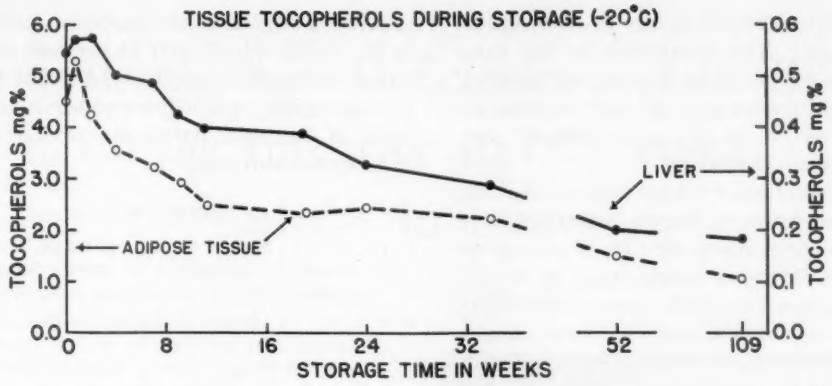

Fig. 1

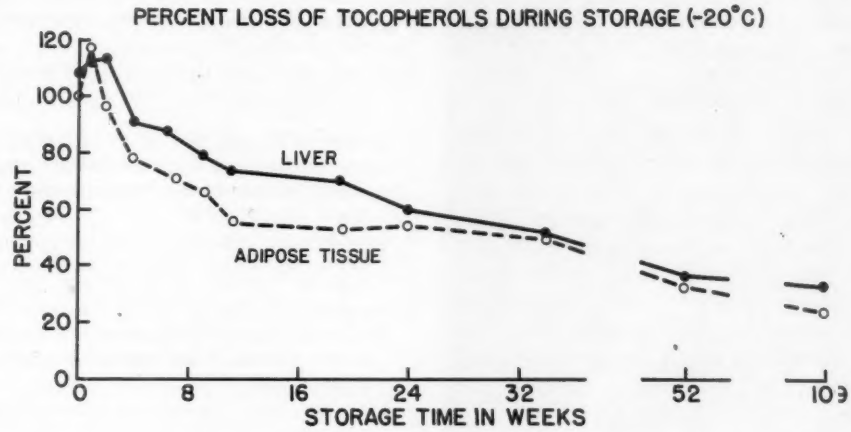

Fig. 2

± 1.3 and 69.7 ± 3.6 for adipose tissue fat, and 94.0 ± 2.0 and 94.7 ± 1.9 for liver fat, respectively; peroxide values of both fats were low throughout this period of storage, usually fluctuating within the range of 0 to 20–30 meq/kg of lipid. Thus neither storage nor molecular distillation appeared to produce any significant change in iodine number or peroxide content of the lipids in adipose tissue or liver.

DISCUSSION

Tocopherols are recognized as important antioxidants in both plant and animal tissues. Although ascorbic acid may have a similar function in animal fat[6] antioxidants other than tocopherols are not deposited to any appreciable extent in animal fat depots under the usual dietary conditions.[7] Even in animal fat, tocopherols are not so abundant but what stabilization can be improved by further addition of tocopherols, or other antioxidants such as ascorbic acid which can act synergistically with tocopherols.[8] In the lipids of human adipose tissue such synergistic antioxidants are probably absent or present in negligible amounts, and tocopherols per unit of fat are at a considerably lower concentration (usually one-half to one-fourth) than in other tissues and organs.[1,2] Such differences, combined with variations in the content of oxidative catalysts and lipoxidative enzymes capable of acting at rather low temperatures and the chain length and unsaturation of the lipids themselves, undoubtedly influence the rate and extent to which tocopherols diminish in various tissues and organs. Whether or not

this progressive loss on storage involves alpha-, gamma-, and delta-tocopherols to the same degree cannot be answered at the moment, since in the studies reported here separate determinations for γ- and δ-tocopherols, combined, were not carried out.

The observations recorded indicate that the loss of tocopherols in adipose tissue and liver during the first month or two of storage at deep-freeze or higher temperatures is of sufficient magnitude to justify some correction of analytical values obtained on tissue samples stored for variable periods of time prior to analyses. The rather limited data obtained for other tissues and organs suggest a lesser loss of total tocopherols on storage than in the case of the tissues mentioned.

SUMMARY

Total tocopherols in human tissues were determined chemically after various intervals of storage. During eight days storage at 4° C, tocopherol loss was greatest in adipose tissue, somewhat less in liver, brain and muscle, and least in lung. Removal of skin from an extremity prior to storage hastened tocopherol deterioration in underlying adipose tissue and muscle. After eight weeks storage at −20° C, tocopherol loss in adipose tissue and liver was somewhat less than after eight days at 4° C.

Prolonged storage at −20° C for 4, 9, 24, 34, 52, and 109 weeks resulted in a decline of tocopherol levels to 79, 66, 55, 50, 33, and 24 per cent of original values in adipose tissue, and to 93, 78, 60, 54, 37, and 34 per cent in liver. In neither tissue was there a significant change in lipid content, or in iodine number or peroxide value of the lipid, during the storage period of more than two years.

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Pantothenic Acid Deficiency and Its Effect on the Integrity and Functions of the Intestines

By THEODORE F. ZUCKER, PH.D.*

A PREVIOUS symposium[1] in this series dealt with the biogenesis and metabolic fate of various vitamin factors and their derivatives which play the role of cofactors in enzyme processes. By means of sound chemical procedures it brought order into problems of enzymology, intermediary metabolism and tissue chemistry. As biologic contributions such investigations are uncommonly satisfying and almost tend to dwarf other phases of nutrition studies. There is no reason however for neglecting other approaches. One of these is the study of the more overt consequences of deficiency states in the animal as a whole and attempts to relate the phenomena to the biochemical properties of vitamins.

This report will center on effects of pantothenate deficiency on the intestine. As a tool in exploring these changes we will, among other things, use the duodenal ulcer which in adult rats results from prolonged deficiency. Superficial defects in the intestinal mucosa and congestion and hemorrhage have been associated with a number of deficiencies. The kind of ulcer we are dealing with is more than that. Figure 1 is a photograph of a fixed specimen of the stomach—seen above— and the duodenum—below. The general distortion need not detract from a gaping ulcer crater to be seen characteristically some $2^1/_2$ cm below the pylorus. Figure 2 shows a section of the duodenum with the mucosa uppermost. The mucosa with its deep glands and villi is intact at the two ends of the section. In the center it is totally necrotic and has partly sloughed. This is an early acute ulcer. The two muscle layers are unaffected. They run continuously across the picture. Figure 3 shows a section

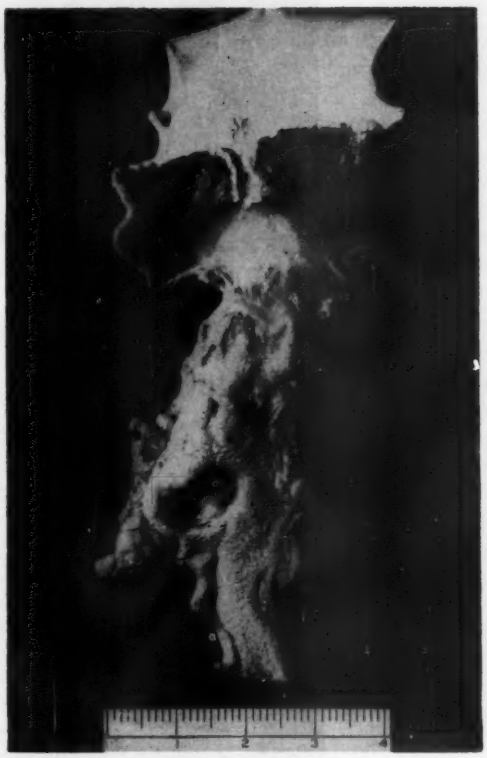

Fig. 1. Photograph of stomach and duodenum of deficient rat showing large ulcer crater in the duodenum.

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through a chronic duodenal ulcer of long standing. Magnification is half that of Figure 2. Only half of the large lesion is shown here. The middle of the ulcer is to the left. To the right the intact mucosa appears as in Figure 2. The muscle layers are below it, more lightly stained. The bulk of the section is composed of a scar which has replaced mucosa and muscularis as well. Grossly this duodenum had been found firmly adherent to the liver, part of which appears, darkly stained, to the right somewhat below the intact segment of duodenum. Perforation of the whole duodenal wall had occurred in the past. The consequent peritonitis remained restricted by the contiguity of the liver but accounts for the large size of the scar. The dark staining areas to the left are pus pockets isolated by connective tissue.

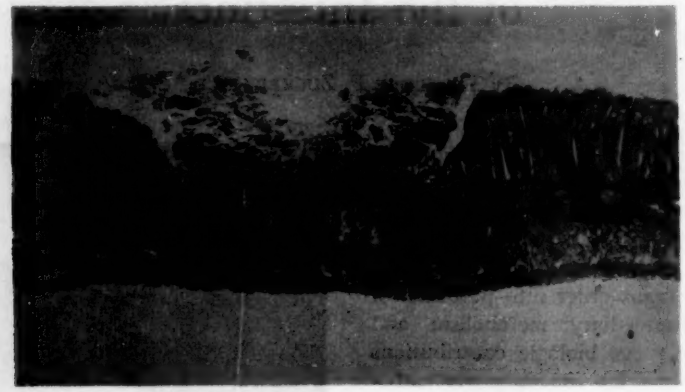

Fig. 2. Section through an early acute ulcer.

Fig. 3. Section through a walled-off chronic ulcer. Magnification is half that of Figure 2.

Even lesions of considerable severity can be cured by pantothenate, leaving a large scar covered by completely regenerated epithelium. These pictures are shown to indicate that this is an unmistakable and serious lesion, as serious to the rat as duodenal ulcers may be to man. At this point however we may as

well curb our interest in analogies of man and rat since due to the wide distribution of pantothenate in foods it is unlikely that there is a common dietary nutritional etiology.

We have shown elsewhere how the effect of pantothenate deficiency can vary with the rat stock used.[2] We will here be principally concerned with two strains bred in our laboratory. Strain differences are nothing new. However, since in a review of our work our strains were represented as inbred,[3] which we stated they were not, a few words regarding them may be in place. We have bred a number of lines for large and small body size by selecting, with avoidance of inbreeding, either large sized or small sized parents in each generation.[4] This has also been done with mice a number of times. (See Falconer[5] for his own work and a review of earlier work by Goodale, by MacArthur, and by Butler). It then usually happens that with the changed genetic character for which selection is made other correlated characters appear. For instance, at the University of Minnesota rat strains were selected for high and low food utilization by Morris.[6] Later Luecke[7] found that these strains differed reciprocally in their requirement for thiamine and riboflavin respectively. In our rats we found that the large and small strains differed in their reaction to pantothenate deficiency. These rat strains have not lost vigor as commonly occurs with inbreeding, and are in no sense abnormal, but simply represent through continued selection the two ends of the variability in some responses to the deficiency. Both strains will react about equally with weight loss, debilitated appearance and ultimate death.

Table I presents data on the large 13C strain and the small 9B strain with ulcer incidences respectively of 54 per cent and 4 per cent. In groups of ten the usual score of the 9B rat is zero, which we have never seen in the 13C strain. Other non-inbred stocks available to us showed two quite resistant ones: the Merck Institute black rat and an albino stock from Food Research Laboratories, Long Island City, New York. A cross of the former with the 13C scored midway between the two. Long-Evans rats from our Anatomy Department turned out to be very susceptible. Their disadvantage is that they rapidly develop an extreme deficiency with many deaths leaving few animals for further experimentation at the time of ulcer incidence.

TABLE I

Duodenal Ulcer in Adult Females

Rat strain	13C	9B
Number of rats	131	45
Per cent incidence	54	4

Incidence tabulated is for large ulcers readily recognizable on inspection of the gross specimen. Many of these are chronic ulcers. A much higher proportion of the rats will show small ulcers whose presence can be fully established only by a study of sections.

The diets were the usual casein sugar diets supplemented with all factors known to be required by the rat except pantothenic acid.[22]

We raised the question whether in pantothenate deficiency a low acetyl choline content, due to low available coenzyme A, might have a bearing on ulcer formation. Among the etiologic factors held by some to be contributory to human duodenal ulcer are disturbances in the submucosal layers of the intestinal wall robbing the mucosa of proper nutriment. A sharp drop or irregularity in the available acetyl choline might have an effect on vascular or other muscle. Assays of acetyl choline by the frog rectus abdominis method in various segments of small intestine were carried out. The results are shown in Figure 4. The number of rats contributing to each average is shown inside the circles. The intestine of the 9B ulcer-resistant strain showed initially about 0.2 mcg of acetyl choline per gram of moist tissue and this value was not seriously affected by pantothenate deficiency of long duration. The susceptible 13C strain starts out with nearly 0.5 mcg of acetyl choline which in three weeks of deficiency drops to about 0.2 mcg/g and remains at that level. Thus we see that even when there is a high initial level, it never drops below the value at which the intestine can function normally. Confirmation of the fact that the acetyl choline does not drop to dangerously low levels results from experiments with surviving gut segments suspended in Tyrode solution with contractions recorded on a kymograph. The segments from various stages of deficiency

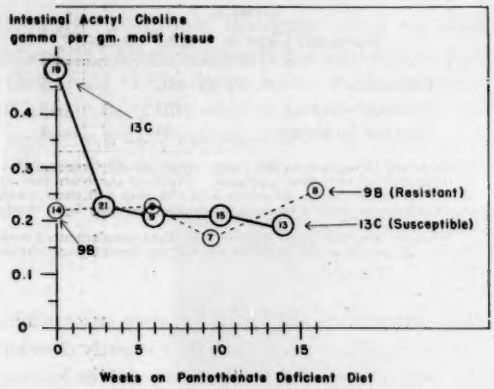

Fig. 4. Intestinal acetyl choline

Intestinal tissue was homogenized in the presence of eserine. After acidification it was placed in boiling water for two minutes. After centrifuging, neutralizing and centrifuging, the supernatant was tested on eserinized frog rectus abdominis using known acetyl choline solutions as standards. Non-eserinized homogenates showed no activity at the dilutions used for assays. Such homogenates when eserine and known amounts of acetyl choline were added gave very satisfactory recoveries of the acetyl choline. Segments from various levels of the intestine in the same rat were in very good agreement.

Variability of the results is high. The difference between the normal and 3-week deficient values for the 13C strain is borderline in significance ($t = 2.6$; value to be equalled or exceeded for 38 degrees of freedom for the 5 per cent level of significance is 2.0, for the 1 per cent level 2.7). The difference between the normal 13C value and *all* 13C-deficient rats has a significance ratio of 6.5.

Isolated gut segments (see text for results) were observed after feeding by stomach tube 3 cc of a 1:3 water suspension of the diet the animal had been receiving. The pieces observed were taken below the usual ulcer sites.

showed as good viability, reaction to drugs, and amplitude of contraction as the controls. Neither the initial drop in the 13C rats nor any other phase of this part of the study has led us any nearer to any direct explanation of ulcer formation. Reasons for a drop in one strain and not in another remain obscure. Balfour and Hebb,[8] however, find two enzyme systems involved in acetyl choline synthesis which differ remarkably in coenzyme A requirement. Several other reports to be mentioned later also indicate that some tissues may hold onto, or conserve, coenzyme A.

As cases of duodenal ulcer in cortisone-treated patients multiplied in the literature, we asked ourselves whether the adrenal might play a role in the experimental rat ulcer. Winters *et al.*[9] have demonstrated low adrenal activity in young pantothenate-deficient rats suckled by mothers which were also deficient. Ershoff *et al.*,[10] also using young rats, produced evidence that under less severe conditions there is no loss of adrenal cortical function. Our animals were adult. When they have been on the deficiency-inducing diet for 11 to 14 weeks the adrenals do show congestion, sometimes hemorrhage, but not much necrosis. Table II shows the relation of the adrenal to ulcer incidence. In 22 rats adrenalectomy

TABLE II

Relation of Ulcer Formation to the Adrenal Gland in Pantothenate Deficient Rats

	No.	Weeks deficient	Ulcer incidence
Adrex	22	$13\frac{1}{2}$	0
Adrex + 1 mg hydrocortisone/day	7	13	57
Hypophysex	5	$14\frac{1}{2}$	0
Medullex	6	$14\frac{1}{2}$	50

All operations were performed before the deficient diet was started.

Adrex (adrenalectomized), and adrex + hydrocortisone: Rats were maintained on salt (1 per cent in drinking water). Some of the adrex rats also received 0.1 mg desoxycorticosterone acetate daily, injected. Hydrocortisone was given in the food, and was started at the tenth week of deficiency.

Hypophysex: Hypophysectomy operations were done by Mrs. Fay Agate, Anatomy Department, Columbia University; rats were checked for completeness of extirpation at autopsy by Dr. Frederic Agate. They were kept in heated cages. A special deficient diet was devised for these rats, to control the extensive diarrhea observed on diet 2517; the new diet had 5 per cent bone ash, 14 per cent cellulose and 10 per cent fat. Intact controls on the same diet developed the usual ulcer picture.

Medullex: In medullectomy half of one adrenal was transplanted to the ovary and remaining adrenal tissue removed. Rats were maintained on salt water for two weeks, tested for functioning cortical tissue by removal of salt, and then started on diet.

reduced the incidence of ulcer to zero, while of seven similar rats given 1 mg hydrocortisone per day for their last three weeks on the deficient diet, four showed ulcers. Five animals with hypophysectomy confirm a role of the adrenal. The medullectomy experiments show that it is cortex and not medulla with which we are concerned.

A commonly observed consequence of pantothenate deficiency—porphyrin-caked whiskers and nose—displays the same relation to the adrenal as does the ulcer; it is prevented by adrenalectomy, not by medullectomy, and is precipitated in adrenalectomized, deficient rats by cortical hormone. However, two other consequences of the deficiency—weight loss and reduction in total bodily acetylation—are *not* affected by adrenalectomy.

To obtain independent evidence as to whether the adrenals of 11-week deficient rats could produce reasonable amounts of cortical hormone experiments on thymus

weight were carried out. It is well known that weight of the thymus decreases under the influence of cortical hormone or stress and that adrenalectomy prevents this. In Figure 5 the upper line shows the normal age involution for a period of 14 weeks at the age we are concerned with (approaching one year). Start-

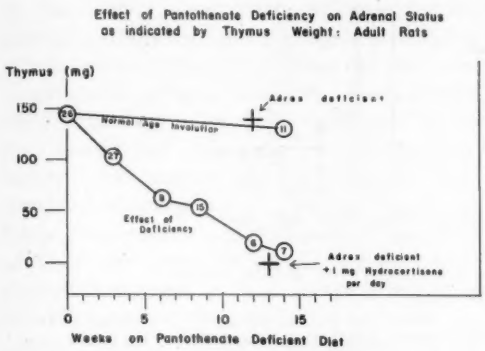

Fig. 5. Effect of pantothenate deficiency on adrenal status as indicated by thymus weight in adult rats.

13C adult females (ulcer susceptible strain); average starting age for different groups 250–330 days; average initial body weights for different groups 260–300 gm.
Standard deviation of thymus weight in different groups ranged from 20 to 46 mg. The difference between the 26 normals and the 27 three-week deficient rats has a significance ratio (t) of 4.5. The difference between the 11 normal age controls and the 13 long-time deficient rats (combined 12 and 14 week groups) has a significance ratio of 10.8. The adrenalectomized deficient group does not differ significantly from the normal; the adrenalectomized deficient group receiving hydrocortisone does not differ significantly from the intact deficient; the two adrenalectomized groups differ very significantly from each other.

ing from a mean thymus weight of about 150 mg the effect of the deficiency is shown in the downward curve ending with a thymus weight of about 10 mg after 14 weeks. That this decrease in thymus weight is not an effect of general malnutrition is shown by the fact that adrenalectomized, deficient rats have a thymus weight normal for their age (upper cross). When, however, 1 mg of hydrocortisone per day is given to adrenalectomized rats from the tenth to thirteenth week of deficiency, the thymus weight is essentially the same as in intact, deficient rats (lower cross). Lymphocyte counts dropping from a mean of 10,400 per cu mm to 3,700 in 11 weeks of deficiency, and eosinophils from 131 to 53 per cu mm, give further evidence of what *appears* to be greater than normal corticoadrenal activity. In the face of all that has been written concerning the destructive effects of pantothenate deficiency on the adrenal, there is certainly cause for considering these data critically.

One of the first questions which arises is whether we should expect or can demonstrate, any excess of adrenal hormone under these conditions. Figure 6 throws light on this question. We have here two separate parallel experiments on thymus weight: one on the complete, the other on the deficient diet. Because we wanted maximal changes in thymus weights these experiments were done on young animals (initial age three weeks) studied for 30 days. The three left-hand columns in each experiment refer to adrenalectomized animals. The first, i.e., highest, column gives the mean thymus weight of adrenalectomized animals on the complete and deficient diets respectively. Since the adrenal is absent, both columns represent the undepressed thymus weight. The second column in each experiment shows the effect of 0.1 mg of cortical extract (Upjohn) given daily during the 30 days. Here we see that in the deficient animals the depression of thymus weight is much greater than on the non-deficient diet. With 0.2 mg the difference between deficient and non-deficient animals is still greater. This indicates that in pantothenate deficiency the thymus is more sensitive to adrenal effects than in non-deficient rats. Body weight differences are far too small to account for the results. It appears therefore that such increase in sensitiveness or vulnerability is one of the important and basic consequences of pantothenate deficiency.

The concept of sensitization of tissues by nutritional deficiencies is by no means new. Ascorbic acid deficiency produces a state of fragility in blood vessels so that minimal injury results in hemorrhage. It is even more clearly demonstrated by experiments of Follis[11] using immobilization of one hind leg of guinea pigs. He concludes that if one eliminates the usual stresses and strains that accompany motion of the extremities many of the classic signs of scurvy fail to appear in the bones.

In Figure 6, the right hand column in each experiment shows the thymus of the intact

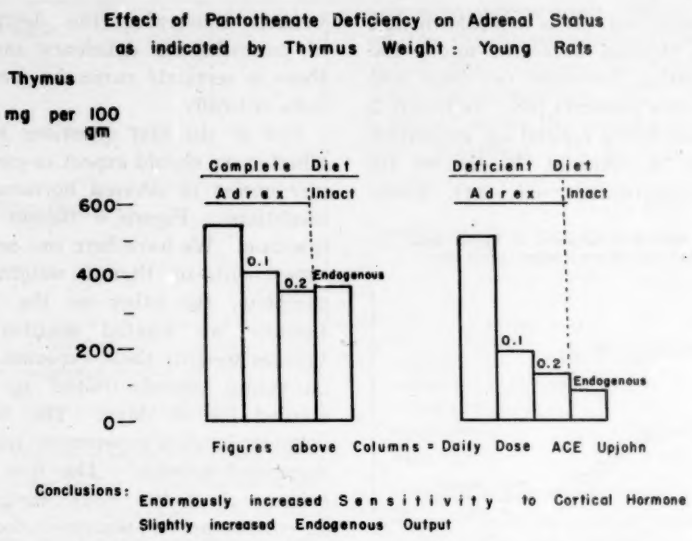

Fig. 6. Effect of pantothenate deficiency on adrenal status as indicated by thymus weight in young rats.

13C females (ulcer susceptible strain); starting age three weeks, experimental period 30 days; diets 2517 (pantothenate deficient) and 2520 (complete); ACE—adrenal cortical extract Upjohn—administered subcutaneously twice daily; all adrex rats given free choice of 1 per cent and 3 per cent salt water, all intact rats given free choice of 0 and 1 per cent salt water. Body weights were initially 45–50 g, finally about 100 g for the deficient, 140 for the complete diet; adrex rats without hormone treatment were about 15 per cent smaller than intact or ACE treated adrex controls. Stock rats covering the body weight range 50 to 150 g show no trend in values of thymus/100 g body weight, with an average value for 21 rats of 400, standard deviation 41. Shortly after the period of the experiment, age involution would normally set in.

Standard deviation of thymus values in various experimental groups ranged from 30 to 60. The number of rats was six to eight except for the two groups receiving 0.2 mg ACE, where N was 3 each. This means that differences of 100 or more in mean thymus/100 g are highly significant. Thus the difference between normal intact and deficient intact (370 − 80 = 290) has a significance ratio (t) of 20.7. The difference due to diet between the 2 adrex groups receiving 0.1 mg ACE (410 − 190 = 220) has a significance ratio of 8.5. The effect of supplying 0.1 mg ACE to adrex rats on the complete diet (540 − 410 = 130) has a significance ratio of 4.1.

rats. The effect of the endogenous hormone is in both cases equivalent to about 0.2 mg adrenal cortical extract. The output of the deficient rats may be a little larger but we have not established a significant difference.

These experiments suggest that what appears to be hyperactivity of the adrenal is not so much exaggerated hormone output as a more sensitive target organ.

In connection with the persistence of steroid production in the adrenal under conditions of pantothenate deficiency we should consider reported studies on sterol synthesis in the liver. Klein and Lipmann[12] have reported that in young rats on a deficient diet for five weeks some livers show low coenzyme A content and, as measured by administration of isotopically labeled acetate, a low rate of cholesterol synthesis. However, others in the group show a practically normal coenzyme A content and correspondingly good rates of cholesterol synthesis. All these animals showed the same definite outward signs of deficiency. Lata and Anderson[13] kept mature (i.e., 60-day old) rats for five months on pantothenate deficiency. They found, also using isotopic acetate, that the synthesis of cholesterol in the liver was even a little higher than in normal controls. Smith and Mefferd[14] reported that cholesterol synthesis during the first 15 minutes after injection of isotopically marked acetate was higher in pantothenate-deficient rats than in controls. The total synthesis during a period of two hours became about equal. Coenzyme A may under varying conditions distribute itself differently among several

available acetylating apoenzymes with the highly essential processes having preference. We have also called attention to individual, strain and age differences in reaction to pantothenate deficiency.

We have presented evidence to show that at the time that duodenal ulcers appear in adult pantothenate-deficient rats the adrenal cortex is functioning, and that without adrenal glands no ulcers appear. This again established *a* link between the experimental rat ulcer and observations on human subjects. As mentioned above, the data as reviewed by Gray[15] leaves no doubt that cortisone or corticotropin therapy has exacerbated or even produced duodenal ulcers in man. The experiments of Gray, Benson, Spiro and Reifenstein[16] and those of Shay and Sun[17] in human subjects have shown that stress by way of the adrenal, or administration of adrenocortical hormone, increases gastric acidity. In Table III we present data on the gastric juice six hours after pyloric ligation (Shay technic). First we have

TABLE III

Gastric Juice, Six Hours after Pyloric Ligation; Adult Females

		No.	Volume ml	pH	Free acid (ml 0.1 N)
13C (ulcer susceptible)	2520 (complete)	11	5.9	1.73	2.3
	13 weeks on 2517 (deficient)	8	9.4	1.35	6.5
	Effect of hydrocortisone, 1 mg/day for 4 weeks, complete diet 2520				
	Intact	8	7.5	1.42	4.9
	Adrex	6	4.8	1.68	1.7
9B (ulcer resistant)	2520 (complete)	7	6.2	1.44	3.7
	13 weeks on 2517 (deficient)	5	6.1	1.31	3.9

This is the Shay procedure for measuring resting secretion. The rats were fasted overnight before the operation, and no water was given after the operation. The period chosen, six hours, is not long enough to produce gastric ulceration (forestomach); the occasional animal with blood in the stomach from fundic bleeding was omitted from the tabulation. The pH is that of undiluted contents. Free acid was obtained by titration with NaOH, the end point being pH 2.8 with a correction made for dilution.

The difference between 13C deficient and normal groups for all 3 tabulated quantities is statistically significant ($t = 5.1$ for free acid, 3.7 for pH, 2.8 for volume).

data on the 13 C susceptible strain. Comparing rats which have been on the deficient diet for 13 weeks with their controls we see that the volume of gastric juice is increased (from 5.9 to 9.4 ml), the pH is lowered (from 1.73 to 1.35) and the free acid is increased (from 2.3 to 6.5 ml of 0.1 N). In the 9B ulcer-resistant strain 13 weeks of deficiency does not produce a noticeable change in gastric secretion.

Table III also shows the effect of daily administration of hydrocortisone to the ulcer-susceptible strain on a complete diet. When these animals had intact adrenals an increase of volume and acidity above that of the controls was found. If the animals were adrenalectomized, hydrocortisone brought the secretion up to the normal range.

The rise in gastric acidity due to cortical hormone in the normal rat, while similar to that produced by the deficiency, is not in itself capable of inducing ulcers. We have given large numbers of normal rats cortisone or hydrocortisone in large doses for up to 17 weeks, or in very large doses, large enough to kill, without producing ulcers. The moderate amount of endogenous hormone in the deficient rats *does* produce ulcers. This also finds a ready explanation in an increased vulnerability of the rat's intestinal mucosa in pantothenate deficiency.

Since the ulcers are a late occurrence (as a rule not before the eleventh week of deficiency) it is of interest to know when a change in gastric acidity occurs. To follow the development of this deficiency sign we devised a method which employed an insoluble complex of the dye Azure A with an exchange resin, Diagnex.®[18] As this is exposed to gastric acid the dye is liberated and absorbed. Its excretion in the urine is taken as a measure of acid secretion. In Figure 7 the data on several series of rats are given as increment of excreted Azure A over the mean initial value for each group. For $3^1/_2$ weeks no change appeared. Then there was a moderate drop. From eight weeks on there was a continuous rise above normal until the animals died or were autopsied. Apparently the ulcers appear only when the gastric acidity has risen to high values. In human subjects made pantothenate-

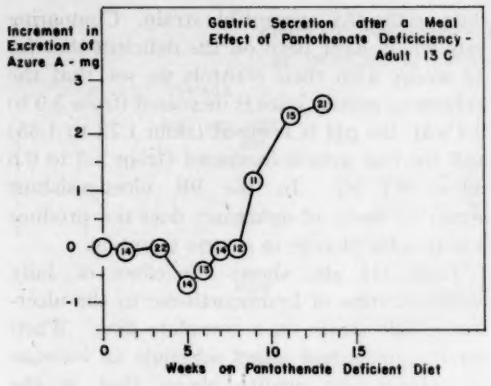

Fig. 7. Gastric secretion after a meal; Effect of pantothenate deficiency in adult 13C females.

Rats were fasted six hours, then given a meal by stomach tube containing 0.8 g dextrin, 0.4 g. gluten, 0.2 g. Diagnex in 5 cc aqueous suspension. This particular food mixture was found to keep the Diagnex sufficiently well suspended to allow quantitative administration. The figures represent 17 hours urine collection, and are the difference between observed value and normal values. Normal absolute values run 3 to 4.5 mg Azure A, so that at the height of the deficiency the output is raised about 60 per cent.

The rate of stomach emptying would be expected to affect the yield of Azure A. Three hours after administration of this meal, seven 13C adult females on 2520 still retained 25 per cent of the test meal solids in their stomachs, while seven rats on the deficient diet for 14 weeks had only 13.5 per cent. Since the deficient stomachs empty faster than the controls, it would appear that if anything the gastric secretion of the deficient rats relative to the normal is *under*estimated by this method.

Both the drop at five to six weeks of deficiency and the subsequent rise are highly significant. Thus a drop at five to six weeks was found in all but two of the 27 observations, made on 19 rats; for the combined five and six week groups t, the significance ratio of the drop, is 5.3.

deficient Bean[19] has recorded only low gastric acidity. It is unlikely that Dr. Bean's volunteers could be carried to the severe deficiency state represented by our rats. This then is another point where analogies between the experimental ulcer and its spontaneous human counterpart break down. With what we know concerning the distribution of pantothenic acid in food it is rather obvious that a dietary pantothenic acid deficiency in man is not likely to be as common as are duodenal ulcers.

It may appear pointless, therefore, even to mention the ulcerogenesis in man in connection with these rat studies. There are reasons, however, why the rat studies may have significance in human medicine. We should recall at this point the story of experimental rat rickets which is produced by a low-phosphorus, high-calcium diet, while human rickets is basically attributable to lack of sunshine. Still it was the experimental rat rickets which gave the impetus to the clarification of infantile rickets which resulted in rickets being no longer a major pediatric problem.

The nature of the action of adrenal hormone on gastric acidity needs to be clarified. This refers to studies both in man and in rats. Gastric secretion is largely under parasympathetic (vagus) control. Sun and Shay[20] have presented evidence that increased gastric secretion in man produced by stress by way of the adrenal does not act through the vagus but *is* suppressed or minimized by anticholinergic drugs. Trials with such drugs in rats support this. Is the corticoadrenal hormone itself a parasympathomimetic or cholinergic agent, or does it lead to the production or release of such a substance?

This brings to mind one of the apparent paradoxes of pantothenate deficiency in rats. Under conditions of low coenzyme A one would not expect a heightened acetyl choline synthesis, and we have not found this. But the excess excretion of porphyrin from the Harderian gland is to the pharmacologist a typical cholinergic phenomenon which he utilizes in assaying anticholinergic drugs or anticholinesterases.[21] Cholinergic and anticholinergic substances are known for marked selective preferences for particular target organs. The release of a cholinergic agent which preferentially affected the Harderian gland would satisfactorily account for the porphyrin excretion of pantothenate deficiency and possibly a number of other findings.

It may be rewarding to study systematically the histologic changes which precede the actual appearance of the ulcer. This would have value as a pantothenic-acid study rather than as an ulcer study. It would also lay a foundation for alternate contemplated chemical approaches to the topic of sensitization.

We can summarize the main points which our model of duodenal ulcers illustrates regarding pantothenic acid deficiency as follows:

Using non-inbred animals which like human beings are, so to say, a mongrel race, i.e., not genetically pure, differences regarding panto-

thesis acid deficiency in individuals or breeds can be established. Deficiency signs differ materially in young and adult rats.[23] Only in the adult rat are duodenal ulcers a pronounced feature. Even after prolonged deficiency the adrenocortical function of adults can still play a decisive positive role. A state of sensitization or increased vulnerability of tissues to cortical hormone appears to play a cardinal role in the deficient state.

ACKNOWLEDGMENTS

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Thanks should also be expressed to the following: Hoffmann-La Roche Inc. for supplies of the various vitamins; The Upjohn Co. for the adrenocortical extract; Merck and Co. for cortisone and hydrocortisone; E. R. Squibb and Sons for Diagnex (improved).

The work was a group project. Concerned in it as much as the present author were Dr. Lois M. Zucker, Dr. B. N. Berg, Dr. Joseph Seronde, Jr., and Miss Catherine Dumbra. Thanks for technical help are also due to Mr. Thorn Many.

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Erratum

In the article "A Formula for Estimating the Specific Gravity of the Human Body with a Consideration of Its Possible Uses" by George R. Cowgill, Ph.D., *Am. J. Clin. Nutrition* 5: 603, 1956, Equation III should have read:

$$\log \text{Specific Gravity } 0.848\ (0.242 \log H_{cm} - 0.1 \log W_g) - 0.0172$$

Editorial

Copper in Health and Disease

One "trace" element which, until recently, has received comparatively little attention in relation to human disease is copper. This substance is found widely distributed in nature and is present in foodstuffs and water, the quantity depending on the soil content of copper. Foods known for their high copper content include nuts, dried legumes, cereals, dried fruits, poultry, fish, and animal tissues. The normal diet contains at least 2.5 to 5 mg of copper per day. The exact daily requirement for copper is unknown but it is of the order of 2 to 3 mg per day in adults. There are approximately 100 to 150 mg copper in the human body. Almost half of this is present in muscles; the bones and liver also contain substantial proportions.

The full significance of the functional role of copper is obscure, but it is known that this metal is concerned in erythropoiesis and that it is important in the formation of bone as well as in maintaining the myelin of the nervous system.[1] Copper deficiency, experimentally produced, results in the development of a severe anemia which, in swine, is microcytic and hypochromic in type and is accompanied by a profound disturbance in iron metabolism. Thus, there is marked hypoferremia, the absorption of iron is impaired and the production of red corpuscles is disturbed. Furthermore, it has been found that in deficient animals a marked reduction in osteoblastic activity takes place. Bone is not deposited on the calcified cartilagenous matrix and deformities develop.[2] Finally, in the syndrome of "enzootic ataxia," which has been described in lambs feeding on a soil deficient in copper in Australia, degenerative changes are found in the corpus callosum, in the internal capsules and in the white matter of the frontal lobes as well as in the motor pathways of the spinal cord.[1]

Copper is also known to be essential for maintaining the color of fur and for the production of "ink" in the squid and octopus. In addition, it is necessary for the folding of the keratin molecule and is thus essential in maintaining the normal structure of wool.[3] Copper is a constituent of certain enzymes and its importance in metabolism is attributable in part to this fact.

In man, considerable interest has been directed recently to the measurement of serum copper and to the variations which occur in health and disease.[4] The serum copper consists of two fractions. One of these, which reacts promptly with diethyldithiocarbamate and is therefore known as the "direct-reacting" fraction, consists of copper loosely bound to albumin and is probably copper in transport. This fraction represents very little of the serum copper. The major fraction, as much as 96 per cent in man, is firmly bound to an alpha$_2$-globulin and consists mainly of the blue copper compound, *ceruloplasmin*. This substance has been shown to possess enzymatic activity as a polyphenyl oxidase. There is good correlation between the oxidase activity of plasma and the ceruloplasmin level but whether or not this is of physiologic significance is unknown. In blood, copper is also found in the red cells. A colorless copper compound which has been named "erythrocuprein" has been isolated recently.[5]

The serum copper is increased under a great variety of circumstances.[1] An increase occurs in pregnancy and is observed also in association with many infectious diseases, when tissue destruction is taking place, in acute leukemia and in many other disorders, including even cases of schizophrenia. It would appear that hypercupremia is a rather nonspecific reaction and therefore, the finding of hypercupremia is of little use as an aid in differential diagnosis.

Hypocupremia, on the other hand, is uncommon. Best known is the hypocupremia of Wilson's disease.[6] In this condition there is a

marked reduction in the ceruloplasmin level. It is not always appreciated, however, that the direct-reacting fraction of the serum copper is not reduced in Wilson's disease and often it is substantially increased in amount. Thus, although in most patients with Wilson's disease hypocupremia is present, the *total* serum copper may, in some cases, be little or not at all reduced.

Hypocupremia has not been observed in human nutritional deficiencies and, in fact, nutritional deficiency of copper has, so far, not been recorded in man. Hypocupremia has been observed, however, in some cases of sprue and it is frequently found in association with the nephrotic syndrome.[7] In the latter condition, the excretion of copper in the urine is correlated with the proteinuria characteristic of this disorder.

Of special interest are the recent reports describing infants who have been observed to have hypocupremia in addition to hypoferremia, hypoproteinemia, edema and hypochromic microcytic anemia.[8,9] The cause of this striking syndrome is obscure. Dietary deficiency of copper and abnormality of protein metabolism at the cellular level have been suggested as the underlying causes. The condition has been corrected rather easily with iron therapy and "spontaneous" cure has also been observed.

Further studies of the role of copper in human metabolism as well as elucidation of the manner in which copper functions in the mammalian organism should be both interesting and fruitful. —M. M. WINTROBE, M.D.

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Diet Therapy

Emergency Feeding in Disaster

By DOROTHY L. BOVEE*

A PROGRAM for the emergency feeding of victims of a major natural disaster calls for large-scale planning, but feeding in the event of an enemy attack requires even more planning, because it presents additional problems on a greater scale of magnitude. However, the goals of emergency feeding are the same in any type of disaster—to keep people alive, to restore and maintain morale, and to provide adequate and familiar food that will keep people at work or enable them to return to work.

Feeding is one of the vital relief services needed in almost every disaster. Those who have had the experience of being the first to arrive at or near a devastated area with food and drink know the immense psychologic lift that the sight of familiar food has upon both disaster victims and workers. It has been amply demonstrated that immediate provision of warm food and drink following a disaster helps to relieve tension, strain, and fatigue, allay feelings of anxiety and fear, and boost morale. The American National Red Cross, through long experience in natural disasters, makes every effort to get feeding operations under way as quickly as possible after disaster strikes, if only to provide a hot beverage and a simple snack in the immediate emergency phase.

While the mechanics and details of feeding are not the physician's province or responsibility under normal or emergency conditions, it is possible that under grave emergency conditions he may be the only trained person present to give on-the-job guidance and direction in matters pertaining to medical, nursing, and dietary services. It is with this very real possibility in mind that many of the details of emergency feeding are included in this paper.

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RESPONSIBILITY FOR EMERGENCY FEEDING

The Federal Civil Defense Administration and the American National Red Cross each have varying responsibilities in natural and enemy-caused disasters. Both agencies have concern for adequate community preparation to meet and cope with disaster emergencies, and to that end they work together in an attitude of cooperation and mutual assistance.

Since its founding in 1881, the Red Cross has, by tradition and the authority of its congressional charter, acted in providing neighborly help to individuals and families suffering from the effects of such natural disasters as hurricanes, floods, tornadoes, fires, explosions, and similar catastrophes.

Government responsibility in natural disasters is in general the same as in normal times—the protection of life, public health, public welfare, and property and the maintenance and repair of roads, bridges, schools, government buildings, other essential public facilities, and public property. By presidential order the administration of government relief responsibilities in natural disaster was vested in the Federal Civil Defense Administration shortly after its creation in 1950. This was in addition to that agency's full responsibility for all disaster relief activities in

the event of enemy attack or the threat of attack on the continental United States.

Responsibility for emergency feeding in natural disasters rests with the Red Cross and is administered in a local chapter by the subcommittee on feeding of the chapter disaster committee. In enemy-caused disaster, emergency feeding rests with civil defense and is carried out by the welfare services.

PROBLEMS IN TIME OF DISASTER

Disruption of the food, fuel and water supplies, sanitation facilities and the communications and transportation systems, places a great burden on the total feeding resources of a community. It is difficult to conceive of preparing and serving meals without these essential utilities and services. Yet in every major disaster some or all of these conditions are encountered and they constitute major problems for those responsible for feeding. Homes may be physically intact but people cannot cook or wash dishes in them if there is no water or fuel. Red Cross disaster experience shows that in emergency mass-care shelters there are always more people to be fed than housed.

There are always those with special food needs—infants, small children, pregnant and nursing mothers, the chronically ill (some on modified diets), and the aged. In addition there are the many disaster workers. Those who help with the rescue and evacuation of the population need to be fed if they are to stay on the job. There may be many injured who have need of care in existing hospitals crowded to capacity even before a disaster. Furthermore, in a disaster resulting from enemy attack with modern weapons, numerous survivors of burns, fractures, massive wounds, other mechanical injuries, and radiation sickness can be expected. Clearly, all those will constitute a major emergency feeding problem.

METHODS OF FOOD SERVICE

In emergencies, food service may be carried out, as it is under normal conditions, by any or by all of the following methods:

Indoor feeding uses local restaurant, school lunchroom, armory, church, lodge, hall, and other functioning kitchens not materially damaged. In areas subject to recurring natural disasters, Red Cross disaster preparedness committees try to earmark buildings that have both adequate kitchen facilities and space for lodging. They make ideal mass-care shelters, and feeding can be done on the premises with the help of personnel who regularly operate the feeding facility. The small restaurant, carry-out, roadside diner, club, pullman car or other eating establishment having a kitchen but limited serving space can be very useful as an auxiliary kitchen. When serving is not required, the volume of food produced for transporting and serving elsewhere can be greatly increased.

Mobile feeding uses improvised or commercially built vehicles to transport, prepare, and serve food. Types of feeding units include the simple mobile canteen, which carries prepared food and drink to the place where it will be served, and the mobile kitchen and convoy, both of which are designed for carrying food, utensils, and supplies with equipment for the preparation and service of simple disaster meals. Mobile operations are usually carried out in the early stages of disaster for workers and victims at or near the scene. Vehicles can move quickly into stricken areas. They are capable of roving over and operating in devastated areas, and of reaching persons in isolated places. Sometimes mobile units rushed in from other communities are, for many days, the only facilities available for feeding victims and workers attempting to restore homes and the community to a functioning level. In most natural disasters of great proportion, assistance from unaffected communities, in the form of mobile support, is almost always necessary. In the event of an enemy attack on one or more major American cities, mobile support on a tremendous scale would be imperative. Every community should plan mutual-aid disaster programs with its neighboring communities.

Outdoor feeding uses kitchen facilities at community picnic or barbecue sites, Army-type kitchens or improvised survival-type outdoor cookers. Contrary to general belief, outdoor emergency feeding is often necessary in natural

disasters. Where no established feeding facilities are available, outdoor feeding stations have to be set up. In the Laredo, Texas, flood of several years ago, 20 army field kitchens and 140 enlisted men were flown in by the Air Force to feed 5,000 flood victims, lodged in 27 Red Cross shelters during the emergency phase of the disaster. Army kitchens and personnel were more recently loaned to Red Cross for the emergency mass feeding of several thousand victims and workers engaged in the rebuilding of Cameron, Louisiana, following the deadly destruction of that city by "Hurricane Audrey."

The construction of such primitive outdoor mass-feeding facilities, as taught by the armed forces, is strictly a survival measure. Conceivably such methods might have to be temporarily used in an enemy atack, but only if there are no conventional facilities, if the people to be fed far exceed the capacity of available conventional facilities, or if people are isolated and forced to function independent of any organized disaster feeding facilities.

It should be kept in mind that in the event of enemy attack, civilians cannot rely upon the military for assistance with disaster relief. Members of the Armed forces will be occupied with the defense of our country. Civilians, then, will have to solve their own feeding problems.

SIMPLE FOOD SERVICE ESSENTIAL

Success in emergency mass feeding in disaster lies in keeping food service simple and practical, even though facilities and equipment for more elaborate meals are available. Meals are usually served cafeteria style, offering a choice of food, even though it be only between kinds of bread and beverages. In the early stages of disaster, if food supplies are limited, menus may have to be planned primarily to allay hunger and sustain morale rather than to fit a rigid nutritional standard. However, in any prolonged disaster situation such as actual war or other national catastrophe, nutrition standards would have to be considered in planning for emergency feeding allowances and the equitable distribution of the available food supply. It follows that menus for such long-term disaster feeding would be designed to provide variety in the diet and to meet individual nutritional requirements as fully and realistically as the food supply permits.

Obviously, disaster is not the time to acquaint people with new and different foods, or to change their food habits. When groups comprised of adults of mixed nationality are being fed in disaster mass-care centers, the foods served should have high general acceptability and should be cooked simply, in ways that permit their ingredients to be easily recognized. Hundley cites studies showing that individuals under stress tend to reject unliked or unfamiliar foods much more readily than they would under normal conditions. In his opinion, this fact rules out the use of bizarre, specially designed food preparations that are often proposed for use in disaster emergency feeding.

In a special report *The Relationship Between Food Habits and Problems of Emergency Feeding* prepared during World War II by the Food Habits Committee of the National Research Council, a number of food items having low emotional value and therefore general acceptability were suggested as best to serve to mixed groups. Among these are:

(1) Plain vegetable or tomato soup, chicken broth with rice or noodles, and split pea or lentil soup.

(2) Beef in all forms, filet of sole and salmon chicken, eggs, and nuts.

(3) Baked or boiled potatoes, boiled rice, spaghetti, noodles, and macaroni.

(4) Green peas, string beans, tomatoes, lettuce, celery, and varieties of raw greens with salad dressing on the side.

(5) Most fruits– fresh, cooked, or dried; ice cream, cookies, and cake.

(6) Coffee and tea; milk for children and those adults who want it.

In the hours immediately after disaster, the first need is for a stimulating beverage, such as steaming hot coffee, cocoa, or tea, or a cold drink such as milk and a simple snack such as a tasty sandwich, crackers, or cookies.

After the immediate emergency stage the need is for a more substantial hot meal. In

a major disaster, mass feeding may have to be carried out on a round-the-clock basis for the first 24 to 48 hours. If the emergency is extreme and the service of a hot meal is not immediately possible, packaged, canned, and fresh foods of known safety that can be served without heating or cooking are provided. When cooking facilities are established, a simple, nourishing, one-dish meal that is easy to heat, transport, serve, and eat is provided.

As soon as means of cooking and serving meals are available, food service can follow the normal pattern of two or three evenly spaced meals a day. In natural disasters, such facilities begin to get organized within a few days, when feeding supplies start to come in and utilities are restored. Every effort is made to terminate disaster mass feeding as quickly as possible and to assist families to return to a normal way of life.

PRECAUTIONS FOR USE OF FOOD

Disaster workers are urged to observe these principles of meal planning under emergency conditions: use only foods that have been rendered usable or declared safe by health officials; avoid foods subject to quick spoilage and bacterial contamination, such as milk, creamed foods, hash, custards, sandwich fillings and salads mixed with mayonnaise, or other perishables, unless refrigeration is available.

Radioactive Contamination

No discussion of disaster feeding is complete without some mention of the hazards of radioactive contamination of food and water following nuclear attack. While the overall safety of food and water in a civil defense emergency is the responsibility of qualified radiological personnel, people can be told that foods in sealed, unbroken packages, cans, or jars will be safe for consumption if the exterior of the container is washed with detergent solution to remove any radioactive substance. Care should also be taken to avoid contaminating contents when removing them from containers. Cooking and eating utensils may also be made safe for use by washing in detergent solution. Wash water and dishcloths must be disposed of by burying. Since boiling does not destroy radioactive substances, special methods are necessary for their removal from water and must be carried out by trained Civil Defense personnel.

SPECIAL NEEDS OF VULNERABLE GROUPS

Aspects of emergency feeding of greatest interest to the physician are those concerned with the foods served to certain vulnerable groups and the conditions of feeding. Infants and children, pregnant and lactating women, the chronically ill and the injured, are groups whose nutritional requirements are normally higher and more exacting or whose food is a normal part of therapy.

Persons assigned the task of feeding disaster evacuees and casualties in mass shelters, emergency hospitals and infant feeding centers should be given some pre-disaster instruction on basic feeding for special groups. Existing hospitals are more likely to have the trained personnel and facilities to handle and supervise specialized feeding.

Infants and Children; Pregnant and Lactating Women (Table I)

Infants and young children are apt to suffer ill effects if deprived of essential foods and liquids for even a short time. Bacterial contamination is an ever present hazard to them.

Stocks of milk and sugar and supplies of safe water must be available for infant feeding within a short time after a disaster. Milk may be either evaporated or dried but it should be stored as should all other formula ingredients, in sealed cans to minimize contamination. The importance of sanitation in every aspect of infant feeding, especially under conditions of disaster, cannot be over-emphasized.

It is important to keep emergency milk mixtures or baby formulae as near pre-emergency proportions as possible. The formulae should be made up under strictly sanitary conditions and kept refrigerated. If normal refrigeration is not available, suitable facilities must be improvised or the formula must be used immediately after preparation.

In addition to any central stations for making infant formulae and other feeding mixtures,

formula ingredients and equipment should be made available, when possible, to those who wish to care for their own children. Because parents are more likely to become panic stricken over the care of their babies than over the loss of homes and property, the practice of parents preparing formulae for their own children is to be encouraged whenever feasible.

In the absence of sufficient safe supplies of fresh fluid milk, concentrated milks would have to be used. Infant feeding should be given priority in available citrus fruit juices and in supplies of uncontaminated water, for the dilution of milk and for formula-making. If water is temporarily unavailable, other sterile fluids such as bottled and canned fruit juices or, in an emergency, even carbonated water might be safely used in formulae.

Sugar or corn syrup is commonly added to infant formulae of diluted whole milk as an extra source of energy. If these are not available, an equal quantity of a more concentrated milk mixture may be substituted, provided the milk (if fresh, whole milk) is modified by heating so that a finely divided curd is formed in the stomach.

The usual practice of warming milk to body temperature may not be possible in an emergency. Safe, unwarmed milk will not harm an infant in most cases, but it should first be given in small quantities to test its effects.

Children from six months to two years of age should receive the same quantity of whole milk as infants under six months, plus enough suitable and available staple foods. Most emergency foods are suitable for children, although some of the food may need proper modification.

Nursing mothers are encouraged to continue breast feeding. If supplies are rationed, they should receive their own and the infant's allotment of milk or a milk equivalent (see Table I) plus the full food and fluid allowances for other adults. If supplies are adequate the same quantity of fresh whole milk or milk equivalent (1 quart daily) is recommended for the pregnant women. Her extra calorie and protein needs can be met in an emergency by providing larger servings of cereals, bread, dried beans and peas, and milk or cheese.

TABLE I

Estimated Ration Requirements for Infants Under Two Years*

Item	Quantity per infant per day	
	Immediately following disaster	Before complete return to normalcy
Water	1 qt	1 qt
Milk, evaporated	1 tall can (14½ oz)	1 tall can (14½ oz)
Sugar, cane	1 oz	1 oz
Crackers, soda or graham	1 oz	—
Cereal, wheat or rolled oats	1 oz	½ oz
Sieved meat or meat soup	—	1—3½ oz can
Sieved vegetable or fruit	—	1—4½ oz can

Recommended Milk Ration for Population

(Grouped in order of priority)

Group	Per person per day *quarts*
Infants, under 1 year	1
Children 1 to just under 2 years	1
Sick children 2 through 9 years	1
Women in last half of pregnancy	1
Well children 2 through 9 years	½
Sick persons of 10 years or over	1
Well children 10 through 14 years	½
Well children 15 through 19 years	½

* Data supplied by Children's Bureau, Federal Security Agency, United States Department of Health, Education and Welfare.

The Chronically Ill

Feeding of the chronically ill who are dependent on modified diets is essentially a medical problem, but disaster feeding teams may be faced with furnishing food to chronically ill persons who are neither hospitalized nor under immediate medical care.

In some conditions, for example, the ambulatory patient with tuberculosis or the patient with peptic ulcer, the temporary disruption of the meal patterns may not cause a serious setback to the patient.

The same is true of the uninjured diabetic on a daily insulin regimen. The diabetic's reserve supply of insulin and the extra syringes and needles to administer it would permit

control of the diabetes even though the diet may have to be obtained from the simple foods available to all.

Butler states that the education of the diabetic should be carried out so that each one will be fully aware of the measures to be taken before an emergency arises. The diabetic should know what to do in the event no insulin is available and he must maintain himself by dietary means alone. A general idea of what he should and should not eat will help. An assured insulin supply is probably more important to a diabetic than any other single factor. Injured diabetics, especially those who may be unconscious, are wholly a medical problem, requiring hospitalization and specialized medical care.

The Injured

Feeding the hospitalized ill and injured is a complicated problem, to be viewed differently from feeding the able-bodied at mass-care centers. This feeding is done at medical sites such as existing hospitals, improvised hospitals, and casualty centers, where the special food needs of patients are under medical control. In an enemy-caused disaster, the provision of food for patients and staff at improvised hospital and casualty stations is designated as a responsibility of the emergency welfare services of civil defense—the same group that is responsible for providing food for disaster workers and the homeless at mass-care centers. In some instances it is conceivable that food personnel who have no previous experience in hospital feeding might have to prepare food and distribute it to the patients. The chance that a well-meaning volunteer might give food to a patient who could be harmed by any attempt at feeding or by being given the wrong food can only be avoided if adequate planning on the part of medical and related services is done in advance of disaster. Any program for feeding the hospitalized injured should be organized so that those responsible for feeding have a way of knowing who should or should not receive food.

It is evident that feeding in hospitals during the acute phase of an emergency must be reduced to simple, manageable proportions. If water, fuel, and fresh food supplies are temporarily cut off or rationed, the normal menu will have to be limited to the serving of such simple food items as may be on hand—canned and packaged soups and stews, meat, fish, milk, vegetables, fruits, juices, and instant beverages. Also, normal tray service may have to be discontinued in favor of bulk service. The use of disposable paper utensils would also be essential, for even though china is available, dishwashing and sanitation might be impossible. Modified diets and infant formulae may also have to be temporarily reduced from a "luxury" level to simple liquid and soft diets and emergency infant formulae.

For high protein feedings, evaporated and reconstituted dried skim milk may be the most practical, inexpensive, and concentrated food sources for use during an emergency.

The following recipes supply approximately the same amount of protein per ounce of feeding or about 150 grams per quart.

Feeding #1	*Feeding #2*
3 cups dry skim milk	3 1/4 cups dry skim milk
3 cups fresh whole milk	1—14 1/2 oz can evaporated milk
	14 1/2 oz water

To mix, measure liquid milk into a bowl or glass jar. Then measure the dry milk powder and sprinkle over the liquid milk. Stir with a spoon or fork, or shake in a covered glass jar, or beat with egg beater if available. Allow foam to settle and refrigerate.

Salt and soda solutions are not a preparation for mass feeding but physicians might need to ask feeding teams to prepare the solution for oral use.

A recipe for the solution:

	For 1 quart	*For 24 quarts*
Stir:	1 teaspoon salt and	1/2 cup salt and
	1/2 teaspoon baking soda into	1/4 cup baking soda into
	1 quart of water	6 gallons of water

STOCKPILES OF EMERGENCY SUPPLIES

Among current FCDA stockpiles are about 13,000,000 intravenous feedings of dextrose and saline and large stores of vitamin-fortified dried skim milk powder, sugar, and minerals. These would be immediately available to

hospitals following enemy attack, for intravenous and tube feeding.

To date Civil Defense has also stockpiled and prelocated two thousand 200-bed improvised emergency hospitals.

Feeding in Deep Shelters

If FCDA undertakes a deep bomb-shelter building program, a new group of feeding problems can be expected to develop around this aspect of emergency care. FCDA officials are currently looking into various types of rations or food packets naturally having, or designed to have a long shelf life. Other factors they must consider in the selection of suitable foods are the need of conserving oxygen, limitations of temperature, humidity, and space which would, no doubt, mean the elimination of all cooking and heating. Rations then, would be designed to sustain life until release from shelters was possible. Problems pertaining to deep bomb-shelter feeding are in the exploratory, early research stage.

OTHER ASPECTS OF EMERGENCY FEEDING

Other equally important areas of emergency feeding are those dealing with (1) prolonged emergencies in which food rationing is essential because of restricted food supplies and (2) feeding workers engaged in essential industries or other heavy occupations, who require a higher than normal food intake. These and other important related aspects of emergency feeding such as general sanitation and the decontamination and safety of food and water, the procurement, transportation, and distribution of food and water; food service equipment and the improvisation of facilities have not been mentioned because of space limitations.

Physicians must be ready to fulfill their role expertly in disaster. The importance of this role is enhanced by the complexity of the present-day international scene and the increasing frequency with which this country is affected by natural disaster of major proportions, making disaster preparedness on a national scale an increasingly important part of our present way of living.

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Nutrition News

Officers of The American Society for the Study of Arteriosclerosis

The newly elected officers of The American Society for the Study of Arteriosclerosis are: President, R. Gordon Gould, Ph.D., Vice President, J. C. Paterson, M.D., Secretary-Treasurer, O. J. Pollak, M.D., Ph.D., Directors, Joseph H. Bragdon, M.D., D. B. Zilversmit, Ph.D., Robert H. Furman, M.D., Ancel Keys, Ph.D., Aaron Kellner, M.D., and Campbell Moses, Jr., M.D. Program Chairman for 1958 is Forrest E. Kendall, Ph.D., Goldwater Memorial Hospital, Welfare Island, New York 17, New York.

American Board of Nutrition Holds Examinations

The American Board of Nutrition will hold the next examinations for certification of specialists in human nutrition, during the week of April 13–19, 1958, at Philadelphia, Pennsylvania. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March 1. Application forms may be obtained from the Secretary, Otto A. Bessey, Environmental Protection Research Division, Quartermaster Research and Development Center, Natick, Massachusetts.

Nutrition Quotes

Nephritic Symptoms from Unusual Beverage

"A remarkable example of prognostic failure was occasioned by a businessman highly successful in his financial dealings, aged 59, limited in activity by virtue of visual defect, happily married, and living in comfort. He developed headaches, drowsiness, thirst, occasional loose stools, nausea, and loss of weight. His doctor, who examined him found that the urine was of fixed low specific gravity (1004); it was loaded with albumin and contained some granular casts. The blood urea was 76 mg. per 100 ml. rising in a month to 110 mg. The blood pressure had risen from his normal of 150/80 to 190/106. The urea clearance test revealed 18% of normal function. He concluded that his patient had chronic nephritis, a view which was endorsed by the consultant to whom he brought the case. In the course of the next three months the symptoms became worse. Albumin was found in considerable quantity in nearly all specimens of urine. A bad prognosis was given. It was thought that the patient would die within the next three months. He was instructed in the traditional lines of dieting for chronic nephritis, and he followed these with care. After a week or two he asked his doctor if the consumption of Worcestershire sauce was in any way harmful. It seemed that he had been taking from half to one bottle of it daily for many years. He said he did so because he liked it. Whatever the reason for his consumption of this enormous quantity, the fact remains that he did take it as a beverage. It was promptly stopped. Within a month the albumin and casts disappeared, the blood pressure and the blood urea had returned to normal, the patient rapidly put on weight. Two years afterwards, he is symptomless, and the only sign of latent renal disease is occasional albuminuria."

—A. H. Douthwaite, *Brit. M. J.* 2: 958, 1956.

Social Custom and Food Habits

"Social custom may dictate a variety of strange food habits. In many countries common foods may be prohibited at certain times, such as pregnancy or lactation, or during illness or mourning; or other unusual foods may be eaten at these times. Insects are an acceptable food in Mexico, Nigeria, and Siam and to the aboriginals of Australia. In Latin America, foods are divided into "hot" and "cold." The former may be consumed at any time; the latter only by healthy adults, but not in the mornings and only if their cold effects are neutralized by hot tea. Foods are also divided into "heavy" or indigestible (for example, rice, milk, dates, figs) and "light" or easily digestible (mutton, chicken, bananas, carrots). Apart from milk, young children are not given heavy foods. Natural sweet foods, that is, fruits, are not compatible with prepared sweet foods containing sugar, and must not be eaten together."

—J. Yudkin, *Lancet* 1: 645, 1956.

Reviews of Recent Books

The Good Housekeeping Book of Baby and Child Care, by L. Emmett Holt, Jr., M.D. Appleton-Century-Crofts, New York, 1957, pp. 288, $4.95.

This attractive book, written by an eminent pediatrician, is a worthy successor to a long line of books known as "Holt's Care and Feeding of Children." Written in a simple direct style, it packs into a relatively small volume a surprisingly large amount of information. Special mention must be made of the many attractive photographs by Tana Hoban and the drawings by Saul Lambert.

The section on feeding is especially well done. The following is typical: "It isn't necessary that every child receive a quart of milk a day, as is sometimes recommended. A pint or a pint and a half will suffice." In the paragraph on poor appetite Holt writes, "The mother is likely to fear that he's starving himself and the grandparents or other members of the household are often even more firmly convinced of this." Thus wisdom is mixed with common sense and extensive experience.

In addition to nutrition, other topics are growth, physical and mental development, behavior problems, and brief discussions of common illnesses. Although written for the parents there is no "talking down." The author recognizes that the modern parent wants more than a dogmatic statement of what to do. The parent also wants to know something of the why. As the author says, he has tried to deal honestly with the common problems of early life. This book can be strongly recommended to all new and expectant parents, because the author's aim has been achieved.

S. O. W.

The Clinical Aspects of Arteriosclerosis, by S. H. Rinzler. Charles C Thomas, Springfield, Ill., 1957, pp. 318, $8.75.

This is a useful, well-documented book. All significant relevant topics are covered and numerous illustrations, some in color, add to the value of the text. The book is somewhat unusual in that it is so thoroughly documented that it often reads as a *summary* of the literature rather than as a *discussion*, based largely on the author's experience. One wishes that there was included more critical comment on the material already published. The section on nutrition is brief but adequate considering the unsettled state of the specialty. All in all this book should prove useful as a review of the current status of a highly complex subject.

S. O. W.

Biochemistry, by Abraham Cantarow and Bernard Schepartz. W. B. Saunders Company, Philadelphia, 1957, pp. 867, $12.00.

According to the authors, this book was designed primarily to meet the needs of the first year medical student. Such a student, if he absorbs and retains a fair proportion of the material presented, would indeed be well informed on biochemistry.

The text is clearly written. The numerous figures, charts and structural formulae, in addition to a good index, enhance the value of the book. The authors go far beyond their original design.

Clinicians, biochemists, and students in allied sciences will find this book an excellent source of pertinent information.

O. M. HELMER

Dorland's Illustrated Medical Dictionary, 23rd edition, ed. by L. B. Arey, Ph.D., Sc.D., W. Burrows, Ph.D., J. P. Greenhill, M.D., and R. M. Hewitt, M.D. W. B. Saunders, Philadelphia, 1957, pp. 1,598, $12.50.

A new edition of this standard work is always welcomed by those whose activities take them into the wilds of medical writing. Meeting the needs of a rapidly growing science the dictionary includes many new terms. Of special interest are the section on modern drugs and dosages by the editor of the *J. A. M. A.* and a section on medical etymology. The long list of distinguished consultants and contributors indicates the reliability of the work. That "Dorland" has been a leading medical dictionary for 57 years is the best recommendation one can make.

R. S. W.

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

Ear, Nose and Throat Dysfunctions Due to Deficiencies and Imbalances by Sam E. Roberts, Charles C Thomas, Springfield, Ill., 1957, pp. 323, $8.50.

A History of Nutrition by E. V. McCollum, Houghton Mifflin, Boston, 1957, pp. 451, $6.00.

Hormones in Blood, edited by G. E. W. Wolstenholme and E. C. P. Millar (Ciba Foundation Colloquia on Endocrinology, Volume XI), Little, Brown & Co., Boston, 1957, pp. 416, $9.00.

Clinical Gastroenterology by Eddy D. Palmer, Hoeber, New York, 1957, pp. 630, $18.50.

Abstracts of Current Literature

FRUCTOSE METABOLISM

An excellent review of the biochemical data concerning fructose has been presented in an editorial discussing the basic reactions involved in its utilization and significant clinical aspects of this agent in therapy. The preferential utilization of glucose over fructose by muscle tissue is an important observation, re-emphasizing the fact that conversion of fructose to glucose is required for complete cellular metabolism of this sugar in extrahepatic tissues.

Clinical Usefulness of Fructose. A. E. Renold and G. W. Thorn. *Am. J. Med.* 19: 163, 1955.

Reactions peculiar to fructose in normal tissues are presented. (1) Fructose can be phosphorylated in position 6 in the presence of hexokinase and adenosine triphosphate (ATP). Brain hexokinase has a greater affinity for glucose than fructose, so that phosphorylation of the latter is inhibited by glucose. (2) Fructose in liver and muscle can be phosphorylated at carbon 1 by a specific fructokinase. (3) Fructose-1-phosphate (F-I-P) is split by an aldolase to two 3-carbon fragments. (4) Glyceraldehyde (one of trioses above) can be phosphorylated by triokinase to phosphoglyceraldehyde. The trioses from F-I-P are identical with those derived from F-I-6-diphosphate and proceed along the chain of glycolytic reactions. (5) F-I-P can also be phosphorylated to F-I-6-P. The liver is the main site of utilization of fructose, taking it up more rapidly than glucose. Fructose is transformed to glucose-6-phosphate which may be released as glucose through phosphatase action. Fructose is poorly utilized by the brain and its administration does not prevent hypoglycemic convulsions in animals. In muscle, fructose utilization is very slow and may be nonexistent. The fructose used by muscle has probably been converted to glucose or other metabolites. Since fructose and glucose are in physiologic competition, studies concerning fructose metabolism have been performed using rat diaphragms with glucose in the medium. In equal concentrations of fructose and glucose the muscle metabolizes 6 to 7 times more glucose in formation of glycogen and $3^1/_2$ times more glucose than fructose in conversion to CO_2. It appears that fructose utilization in muscle is small compared to glucose. Normal fructose tolerance in diabetes suggests that diabetic tissues utilize fructose as well as or as poorly as normal tissues.

Clinically, fructose may be of advantage in treatment of liver disease because of its rapid uptake by hepatic cells and its greater protein-sparing effect. Fructose may represent a superior intravenous sugar because of a lower renal threshold and reduced wastage. Fructose is rapidly utilized by the liver while the action of insulin on hepatic tissue in promoting glucose uptake is delayed so that fructose may be of value in treatment of diabetic acidosis. The functional state of the liver may be assessed by use of the fructose tolerance test.

In patients receiving insulin, the peripheral utilization of glucose may exceed the rate of glucose formation from fructose, and hypoglycemia may develop. With rapid utilization of fructose, sufficient lactate and pyruvate may accumulate to produce acidosis. Rapid glycogen deposition may cause hypokalemia. It is emphasized that further clinical appraisal of the usefulness of fructose is required. C. R. SHUMAN

Since fructose undergoes a series of reactions within hepatic cells to form either glucose by condensation of trioses, or glycogen, the rise of blood glucose levels following its administration is lower—but sustained for longer intervals—than that obtained following glucose.

Blood Sugar Levels Following Intravenous Infusion of Glucose and Fructose in Adults. J. C. Peden, Jr., J. S. Riley, L. Bond, and R. Elman. *Metabolism* 4: 318, 1955.

Twelve normal subjects were given intravenous 10 per cent glucose and fructose on alternate days at a uniform rate. Blood sugar (total reducing) levels were observed during and for three hours following the infusion. The blood sugar levels immediately after the glucose infusion were much higher than after fructose, whereas during the postinfusion period the differences were reversed. Following fructose, the minimal blood sugar levels were similar to the fasting values; the average minimal postinfusion level following glucose was about 20 mg per cent lower than fasting. Hypoglycemic manifestations were observed in one patient following glucose but in none following fructose. Because of the absence of postinfusion hypoglycemia it is suggested that fructose may represent a more desirable carbohydrate for parenteral administration.

C. R. SHUMAN

The substitution of fructose for glucose in the treatment of diabetic keto-acidosis has not provided the advantages ascribed to it by the original reports. The early use of either hexose in the presence of severe hyperglycemia and acidosis combined does not seem warranted.

Fructose in the Treatment of Severe Diabetic Ketosis. J. D. N. Nabarro, J. C. Beck, and J. M. Stowers. *Lancet* 2: 1271, 1955.

The effect of giving fructose and insulin in severe diabetic ketosis was tested in five patients and compared with the effect of routine treatment with glucose and insulin, or insulin alone.

Plasma sugar and ketone levels, CO_2-combining power, and urinary sugar were estimated, and water and electrolyte balances carried out. The cases are documented in detail.

The administration of fructose tended to delay the fall in blood sugar and increased the insulin requirement. Clinical improvement was satisfactory in all the cases; "the ketosis possibly cleared a little quicker in the fructose-treated patients." A significant increase of glycosuria or of osmotic diuresis could be avoided in the fructose-treated group if sufficient insulin was given, but it was more difficult to assess the amount needed.

F. E. HYTTEN

Interrelationship of Acute Alkalosis and Potassium Metabolism. C. R. Kleeman, M. E. Rubini, E. Lamdin, R. F. Kiley, and I. L. Bennett, Jr. *Metabolism* 4: 238, 1955.

Acute methyl alcohol poisoning occurring in a patient was treated by the rapid intravenous administration of 1600 meq of sodium bicarbonate solution, resulting in metabolic alkalosis and potassium depletion. During hospitalization, metabolic data were accumulated on sodium, potassium, and chloride balances and on urinary ammonia, blood and urine pH, titratable acid, and serum organic acids. Rapid renal loss of potassium was detected as the cause of potassium depletion. The potassium loss potentiated the metabolic alkalosis and favored the excretion of an acid urine. Apparently an increased hydrogen ion concentration develops within the renal tubular cells in hypopotassemic alkalosis, favoring the secretion of acid. Coincident with potassium repletion, the excretion of ammonia and titratable acid rose further. The high rate of excretion of ammonia initially with an alkaline urine suggests that factors other than the urinary pH are important stimuli for ammonia production.

C. R. SHUMAN

The Detection of Mucoviscidosis by the Determination of Saliva Chloride. K. McGrady and S. P. Bessman. *Am. J. Dis. Child.* 90: 610, 1955 (Soc. Trans.).

A relatively simple procedure for the measurement of the chloride content of saliva has been applied to the analysis of specimens from 100 children with various diseases and 5 children with mucoviscidosis. The patients with mucoviscidosis had all been diagnosed after repeated examinations of the duodenal secretions.

It was found that the chloride concentration of saliva varied markedly with the method of collection and that consistent results were obtained only with the parotid secretion. This was obtained by placing the pledget of cotton in the patient's cheek and holding it there till it was saturated—a period of five minutes or less.

One hundred patients with various diseases had a range of 6.8–22 meq per liter.

J. N. ETTELDORF

Sweat Electrolyte Studies in Mucoviscidosis. H. Shwachman, R. R. Dooley, and M. Stern. *Am. J. Dis. Child.* 90: 614, 1955 (Soc. Trans.).

Further evidence is presented to show that mucoviscidosis is a generalized disease involving the sweat glands as well as mucus-secreting glands. The excessive secretion of sodium and chloride is of such magnitude that this measurement can be used as a diagnostic test. The information derived from this test is as reliable as that obtained from the examination of the duodenal fluid. Its relative simplicity may make it a more valuable test. A practical and simple procedure for the collection of sweat in infants and children has been devised. (This method is not described in the society transactions.) No other condition has been encountered with an elevation of the sweat sodium and chloride to the same degree as mucoviscidosis. The elevation of electrolytes in mucoviscidosis is independent of the severity of the degree of pancreatic insufficiency. The test is of special value in studying patients with the typical pulmonary picture of mucoviscidosis who have little or as yet no demonstrable pancreatic enzyme insufficiency.

J. N. ETTELDORF

OBESITY

The clinical evaluation of obesity may involve serious errors if one relies upon standard weight charts. The common practice of referring to reference tables to determine the so-called ideal weight for height and age has been demonstrated to be fallacious in a significant number of patients, particularly those with a large lean body mass. These individuals while considerably overweight, according to "standard weight," are not obese, i.e., do not have excessive fat deposition. Likewise, thin persons may have normal lean body mass with limited fat content. Creatinine excretion which reflects the body muscle content has not proved effective as a method of evaluating obesity contrary to expectations.

Creatinine-Weight Coefficient as a Measurement of Obesity. S. M. Garn and L. C. Clark, Jr., *J. Appl. Physiol.* 8: 135, 1955.

The creatinine coefficient is defined as the 24-hour creatinine excretion in milligrams divided by the body weight in kilograms. Low values in this ratio are associated with clinical obesity. Excreted creatinine parallels the size of the lean, fat-free, or metabolically most active body mass. Therefore the creatinine-weight ratio should be inversely related to the amount of fat present. Trochanteric fat and estimated percentage fat showed a moderate negative correlation with the coefficient. Lean men were observed to have high coefficients. However, low values did not pick out obese males. M. J. OPPENHEIMER

Overweight, Obesity, and Coronary Heart Disease. J. Brozek and A. Keys. *Geriatrics* 12: 79, 1957.

The authors again review the concept of overweight versus obesity. Obesity means excessive fatness, and overweight can be either from excess fat or from having greatly developed muscles. They point out that most statistics on coronary disease are based on simple underweight-overweight and not degrees of fatness. Necropsy studies on the relationship between atherosclerosis and obesity are inconclusive. They do prove that atherosclerosis and coronary disease are not restricted to obese individuals. Studies from Finland and Holland during the war years suggest that atherosclerosis may not be entirely fixed once it develops. There is some hope that the lesion may be in some measure reversible.

Heart disease as a cause of death occurs one and one-half times more often in overweight individuals than it does in those of "standard" weight. This is not specifically recorded as being due to coronary disease. The evidence for an increased incidence of obesity in patients with coronary heart disease is lacking. On the other hand leanness does not protect one from this illness.

The relationship between obesity and blood cholesterol seems to depend upon the general level of blood cholesterol in the group under study. In this country where there is generally a high cholesterol, no definite correlation with obesity is found. Among the Bantu in South Africa with a generally low level of cholesterol, the overweight individuals tend to have high cholesterol levels.

The authors feel that weight reduction is not the main answer to reducing the incidence of cardiovascular disease in this country. For instance, cholesterol levels decrease during actual weight reduction but eventually return to the level characteristic of the individual prior to weight change. They feel that the insurance companies' statistics on the benefit of weight reduction reflect not only rerating but also reselection. This may give misinformation as to the benefit of weight reduction.

This is a thought-provoking paper and points out once again that interpretation of statistical data must be carefully analyzed before drawing too many conclusions. K. R. CRISPELL

The difficulty that many obese patients encounter in achieving weight reduction on restricted caloric intakes suggests a metabolic abnormality involving energy production. Elevated pyruvate levels in obesity have been reported to be indicative of a block at the site of decarboxylation of pyruvate. Further investigation as to the cause of hyperpyruvemia in obesity and the metabolic derangements involved are awaited. Weight loss on isocaloric diets high in fat and protein content have been recorded in obese subjects by several investigators.

Blood Pyruvic Acid in Obesity. L. Nyfos and A. P. Skouby. *Acta Med. Scandinav.* 156: 403, 1957.

Blood pyruvate levels were measured both in the fasting state after a light meal and after ingestion of 800 cal, primarily fat or mainly carbohydrate. The results indicate that the pyruvate levels are significantly higher in obese than in nonobese subjects. This is true both for fasting and after a moderate intake of carbohydrate. No correlation was found between the pyruvate values and the degree of obesity, nor was there any evidence that the elevated levels were due to an abnormally slow transformation of pyruvate to other metabolites. S. O. WAIFE

Calorie Intake in Relation to Body-weight Changes in the Obese. A. Kekwick and G. L. S. Pawan. *Lancet* 2: 155, 1956.

It is commonly held by persons who are dieting to reduce weight that small changes in diet, insufficient to affect the calorie intake, have marked influences on weight loss. The experiments recorded in this paper set out to establish the relative importance of calorie restriction and alteration of diet composition in the treatment of obesity.

On diets containing about 20 per cent protein, 33 per cent fat and 47 per cent carbohydrate, loss of weight was proportional to the calorie content. When calorie intake was constant at 1,000, daily weight loss was

most rapid when the diet contained 90 per cent fat, less rapid when it was 90 per cent protein, and weight could be maintained for short periods when it was 90 per cent carbohydrate. Each diet contained 3 liters of water and 10 grams of sodium chloride.

On a daily intake of 2,000 calories, weight was maintained or increased in four out of five obese subjects. In the same subjects significant weight loss occurred when the daily calorie intake was raised to 2,600, provided this intake was given mainly in the form of fat and protein.

There was no defect in absorption of these experimental diets which could account for the weight loss. There was neither loss of body protein stores nor of carbohydrate stores to a degree which significantly contributed to the weight loss. Weight lost on the diets was partly derived from body water, but total body water, measured by a urea-dilution method, maintained a constant relationship with body weight. As the rate of weight loss varied so widely with the composition of the isocaloric diets, it is suggested that obese patients must alter their metabolism in response to the contents of the diet. The rate of insensible water loss was shown to rise with high-fat and high-protein diets and to fall with high carbohydrate diets. This supports the suggestion that an alteration in metabolism takes place, although no change in the basal metabolic rate could be measured.

The results of this enquiry are reported fully; it is an important paper and will repay detailed study.

F. E. HYTTEN

Influence of Body Type and Body Fat Content on the Metabolic Cost of Work. A. T. Miller Jr., and C. S. Blyth. *J. Appl. Physiol.* 8: 139, 1955.

Male college students were made to run on a graded treadmill. It was observed that gross body weight served best to predict the metabolic cost of work or exercise. Less useful were lean body mass and circumference of chest or abdomen. Any value these latter factors had in prediction pertained to their correlation with body weight. The ability to do hard work is limited by obesity. This is so because the energy cost of work or exercise is increased but there is less ability to increase uptake of oxygen.

M. J. OPPENHEIMER

Differences in steroid excretion rates between obese and nonobese subjects may have little significance in the pathogenesis of obesity since storage and metabolism of these compounds may differ in the two groups. A more significant study would be the determination of serum levels of the steroids and their responses to stimulation or suppression.

Steroid Studies in Normal and Adipose Children. C. H. Gray, J. B. Lunnon, M. H. Pond, and S. L. Simpson. *J. Clin. Endocrinol.* 16: 473, 1956.

This investigation was undertaken to determine whether obese children who present certain features resembling those found in Cushing's Syndrome excrete abnormal quantities of adrenocortical steroids or 17-ketosteroids. The steroid analyses done in this study were elaborate as the urinary excretion of compound F, cortisone, tetrahydrocortisone, androsterone, and etiocholanolone as well as others were determined. Measurement of amounts of the various steroids present in urine was only semiquantitative, however, since this was accomplished by visual estimation of steroids present on paper chromatograms.

It was found that obese boys and girls both excreted significantly larger amounts of hydrocortisone than did normal children and also that another steroid (Reichstein's compound U) was present in increased amounts in the urine of obese boys. There was no difference in 17-ketosteroid excretion when the obese children were compared to normal controls.

If there is actually an increase in the adrenal secretion of compound F in these obese children, an important clue to the pathogenesis of this disturbance has been provided. It does not seem likely that such an increase in hydrocortisone secretion is present, however, since the urinary excretion of tetrahydrocortisone, a major metabolite of compound F, was not significantly different in obese children when compared to normal boys and girls.

A. B. EISENSTEIN

The psychiatric studies dealing with obesity continue to be plagued by the problem of the lack of providing a control group of nonobese patients. Definite conclusions concerning the role of emotional maladjustment as a frequent cause of obesity cannot be rigorously supported although isolated instances can always be cited by those interested in this hypothesis.

Fat Children Grow Up. H. Bruch. *A.M.A. Am. J. Dis. Child.* 90: 501, 1955 (Soc. Trans.).

During 1953–1954, follow-up observations were made on former fat children who had been studied from 1937–1940 at the Babies Hospital and the pediatric department of Vanderbilt Clinic. All fat children who had come to the attention of the different clinics at the Medical Center during that period had been included in the original study. Follow-up letters were sent to 113 fat boys, but the majority were returned as unclaimed. Only 42 received the follow-up notices, and of these, 24 young men, or 57 per cent, came for a follow-up visit. Of the 112 fat girls, 32 were reached by a follow-up letter, but 10 responded to it. The following report is based on the later development of the 24 fat boys.

The earlier study had shown that the then current concepts on the endocrinologic etiology of obesity were erroneous. At the same time it was noticed that an intense emotional involvement of the parents, usually the mother, with the child, led to overfeeding and overprotection. The resulting overeating and inactivity gave a seemingly simple explanation for the develop-

ment of obesity. There was strong psychological resistance against changing the faulty habits.

The follow-up study was designed to determine whether the adult adjustment, both in terms of weight and of emotional development, could have been predicted on the basis of the medical and psychological findings in childhood. If one evaluates weight and emotional adjustment as independent variables, four possibilities exist for their combination, namely, that of slenderness with good adjustment, slenderness with poor emotional adjustment, continued obesity with good adjustment, and continued obesity with maladjustment. All four combinations were observed in this group, with the last group having a subdivision of obesity with frank psychotic development. The re-evaluation of early data in the light of the outcome in young adulthood leads to the conclusion that a detailed history of the physical and emotional development of an obese child and his family relationships permits a fairly accurate prediction of the future course.

Therapeutic interference, particularly dietary restriction, did not influence the long-range weight development; in some cases it had become the focal point of serious emotional problems. The effect of endocrine treatment was even poorer. Although psychiatric disturbances had been recognized early in many instances, adequate psychiatric treatment had not been carried out in these cases because of resistance against the intensive work that would have been required for the fat child and the involved parent.

J. N. Etteldorf

Obesity in Women: A Psychiatric Study. M. B. Hecht. *Psychiatric Quart.* 29: 203, 1955.

The author describes some of the psychologic factors operating to produce obesity in women, presenting data obtained from the interviews of 13 obese women. A case history of a woman hospitalized for obesity is presented in detail.

Food gratified the oral needs in these women, which were particularly strong. If they did not eat they would become tense; and tension could be relieved only by eating. Food, also, particularly fattening foods, is unconsciously equated with the penis or breast which the patient would like to devour and make part of herself. Thus, eating represents the gratification of oral eroticism and, at the same time, a sadistic, sexualized attempt to steal or capture the wished-for phallus or breast.

The tremendous guilt felt after eating is derived from the obese patient's unconscious appreciation of the meaning of the act. Periodic abstinences and rigid attempts at dieting represent the ego's effort to prevent further crimes and expiate for those of the past. However, the overwhelming need for gratification leads to renewed gorging.

The obese woman is prevented from losing weight also by the anxiety produced by diminution in body size. She wishes to show to the world that she possesses, on her fat abdomen, those objects that both she and others hold in high esteem. (The author does not state specifically what these objects are, but he is probably referring to the penis). This explains why many women stop dieting as soon as they are told that they have lost weight.

Obesity may be used to defend against threatening unconscious feminine or masculine wishes. Thus, the large breasts and curves are accentuations of feminity, and, therefore, may be an attempt to deny unconscious masculinity. Also, since men are less attracted to obese women, obesity may be a defense against the dangers of being feminine.

Because of these dominating unconscious psychic factors, the obese woman has difficulty in losing weight. It is not possible to understand the nature of obesity without taking into account these unconscious factors.

S. W. Conrad

The mobilization of fat from depots during periods of weight loss associated with severe acute or chronic illnesses is usually uniform for all body areas as clinicians have appreciated for many years. The following observations confirm these impressions.

Fat Changes During Weight Loss. S. M. Garn and J. Brozek. *Science* 124: 682, 1956.

In this study, 13 clinically healthy active young males were placed on a 1,000 calorie diet for 24 days. Measurement of the changes in the subcutaneous fat-plus-skin layer on nine parts of the body was determined by soft-tissue teleoroentgenograms. All of the subjects lost weight: the median weight loss was 8.3 kg, or 12 per cent of the original value. Losses in subcutaneous fat as determined by this technic were clearly related to the initial thickness; thus, the greatest loss occurred from the parts of the body with the thickest fat deposits (deltoid, iliac, and trochanteric regions). These observations indicate that fat is withdrawn in proportion to the initial amount of fat present and that relative fat patterns before and that after weight reduction are preserved.

S. O. Waife

Obesity sufficient to interfere with pulmonary ventilation has recently recaptured the attention of the medical public and has been termed the "Pickwickian Syndrome" after the famous Dickens' character. (*See editorial, this* Journal *5: 344, 1957.*)

Polycythemia Associated with Obesity. M. H. Weil. *J. A. M. A.* 159: 1592, 1955.

Some association between obesity and polycythemia has been reported from time to time. True polycythemia (a hemoglobin persistently over 19 g/100 ml) was found in only 0.8 per cent of 260 obese subjects; nevertheless, this incidence is ten times that found in the general population.

In three cases reported in this paper, the findings differed sufficiently from polycythemia vera and from

polycythemia due to hypoxemia to warrant, in the author's opinion, a separate classification. There was no increase in immature red cells, and no white cell or platelet alteration. The bone marrow was not hyperplastic. Splenomegaly was not found, cyanosis was absent, and moderate doses of radiophosphorus were ineffective. Arterial oxygen saturation was only slightly depressed.

Because significant weight reduction seemed to be curative, the author suggests that this is "a form of secondary polycythemia due to inadequate pulmonary ventilation presumably because of the mechanical barrier created by fat." S. O. WAIFE

The location of cells within the hypothalamus controlling the food intake has been demonstrated by means of a stereotaxic device which permits the destruction of small discrete areas within the brain. These centers of appetite control are regarded by some as being sensitive to changes in rates of glucose utilization. Increased utilization reduces appetite and is manifested by a widened arteriovenous glucose difference; decreased glucose utilization increases appetite and is accompanied by a small A-$\bar{V}$ glucose difference.

Hypothalamic Obesity in the Mouse—Production, Description and Metabolic Characteristics. J. Mayer, R. G. French, C. F. Zighera, and R. J. Barrnett. *Am. J. Physiol.* 182: 75, 1955.

By means of a modified stereotaxic device hypothalamic obesity was produced in Swiss mice and in thin littermates of hereditary obese-hyperglycemic mice. The authors give the co-ordinates for successful bilateral lesions involving the ventromedian nuclei and closely adjacent hypothalamus. Swiss mice were observed to have decreased spontaneous activity and marked hyperphagia. Oxygen use is proportional to surface area. No abnormalities in glucose blood levels, resistance to growth hormone, or sensitivity to insulin were observed in Swiss mice which were obese because of hypothalamic stereotaxic lesions. Similar observations were recorded for nonobese littermates of hereditary obese specimens. If the diet was high in fat, the gain in weight was large. Conversely, if a high protein-diet was used, the gain in weight was small. The authors stress the fact that many factors play a role in the production of obesity. M. J. OPPENHEIMER

The Turnover of Liver Glycogen in Obese Hyperglycemic Mice. K. H. Shull and Jean Mayer. *J. Biol. Chem.* 218: 885, 1956.

Comparisons of the liver glycogen of 25 genetically obese hyperglycemic mice and 26 nonobese mice were made. The diet used prior to and during the experimental period was commercial laboratory chow. Each mouse received a known amount of uniformly labeled glucose-C^{14} by intraperitoneal injection at time of zero and was killed by blow on back of head 0.5 to 4.0 hours later. The turnover of liver glycogen was measured by the amount of glucose-C^{14} incorporated into liver glycogen.

The levels of liver glycogen per gram of liver were similar in the obese hyperglycemic mice and their nonobese controls, but the former group had more than twice as much total glycogen. The greater total glycogen was due to the greater liver mass of the obese hyperglycemic animals. Six male Swiss mice, made obese by gold thioglucose, were studied for comparative purposes. They had livers of comparable size to those of obese hyperglycemic mice and also showed greater total glycogen levels. The glycogen levels of skeletal muscle were significantly higher in the obese hyperglycemic animals than in the nonobese ones.

The turnover of liver glycogen as measured by the incorporation of uniformly labeled glucose-C^{14} was approximately six times as great per total glucose and three times as great per gram of liver and per milligram of glycogen in the obese hyperglycemic mice as in their controls. M. K. HORWITT

Glucose Tolerance in Relation to Obesity and Food Intake. R. A. Bloom and P. F. Fenton. *Am. J. Physiol.* 184: 438, 1956.

Glucose tolerance tests were carried out on two strains of mice, one highly susceptible, the other only moderately susceptible to nutritional obesity. With glucose administered on the basis of body weight or fat-free weight, the highly susceptible strain disposed more rapidly of the administered glucose than did the moderately susceptible strain. Fasting blood sugar levels in both strains were found to increase with the logarithm of body weight. These observations are not readily explained in terms of the "gluco-static" theory of the control of food intake. AUTHORS

Muscle and Liver Glycogen of Mouse Strains Susceptible or Resistant to Nutritionally Induced Obesity. J. B. Lyon, Jr., and P. F. Fenton. *Am. J. Physiol.* 187: 415, 1956.

Muscle and liver glycogen levels of mice differing in their susceptibility to nutritionally induced obesity were studied in relation to inherited differences in metabolic and endocrine patterns. The I/Fn strain, resistant to nutritional obesity, is characterized by a muscle glycogen level four to six times higher than those of strains which can be made obese. The liver glycogen of the I strain mouse is significantly lower than those of the other strains. Muscle glycogen levels were found to reach a maximum at about six months of age in all but one of our strains. AUTHORS

The New Zealand Strain of Obese Mice: Their Response to Stilbestrol and to Insulin. M. Bielschowsky and F. Bielschowsky. *Australian J. Exper. Biol. & M. Sc.* 34: 181, 1956.

This paper describes an inbred New Zealand strain of obese mice. There seem to be significant differences

between this strain and the "hereditary obese hyperglycemic" mice of Mayer. Stilbestrol corrects some of the metabolic abnormalities: it lowers the elevated blood sugar and prevents or reduces the excessive deposition of fat. In this animal, insulin is tolerated in doses otherwise fatal to normal mice. The histology of the pancreas does not indicate hypersecretion of glucagon; in fact, there is a decrease in the number of alpha cells. In the pancreas, there are giant islets full of granulated beta cells, a picture suggesting high insulin production. All in all, the evidence seems to indicate that a state of hyperinsulinism exists in these obese mice and that there is a pituitary factor, not yet identified, which opposes the action of insulin.

S. O. WAIFE

Increased Intestinal Absorption of Glucose in Three Forms of Obesity in the Mouse. J. Mayer and C. Z. Yannoni. *Am. J. Physiol.* 185: 49, 1956.

In three types of obesity in the mouse, obese-hyperglycemic syndrome, gold thioglucose obesity, and hypothalamic obesity, there is observed an increase in intestinal absorption of glucose when the dose of glucose administered is sufficient. The difference with normal animals is due neither to differences in gastrointestinal weight nor to differences in rate of gastric emptying. This phenomenon appears to be an adaptative result of prolonged hyperphagia. AUTHORS

Fat Metabolism in Experimental Obesities. VII. Lipogenesis and Cholesterologenesis in Mice With Adrenocorticotropic Tumors. C. Zomzely and J. Mayer. *Am. J. Physiol.* 187: 365, 1956.

In vivo incorporation of acetate-1-C^{14} into fatty acids and cholesterol by carcass and liver was determined 30 minutes after injection of the labeled acetate in mice grafted with adrenocorticotropin-secreting tumors. Serum cholesterol levels were also determined. Lipogenesis is elevated under fed and fasted conditions in mice bearing adrenocorticotropin-secreting tumors as compared with normal controls or tumor-bearing adrenalectomized mice. Cholesterologenesis was not significantly different in fed tumor-bearing and control mice. When fasted, however, the tumor-bearing mice exhibited greater incorporation of C^{14} than that of their controls. Fasting did not decrease cholesterologenesis in the former group. Serum cholesterol levels of the adrenocorticotropic mice were found to be twice as high as those of controls. In addition, the amounts of carcass and liver fatty acids were three times as great for the ACTH mice as compared with controls, although their body weights did not differ significantly. Carcass and liver cholesterol content of adrenocorticotropic mice was also elevated. The results of this experiment indicate that mice bearing adrenocorticotropin-secreting tumors exhibit a "metabolic" type of obesity. AUTHORS

ATHEROSCLEROSIS: NUTRITIONAL ASPECTS

The sweeping conclusions which have been made concerning the incidence of atherosclerosis and dietary fat are not entirely acceptable. Epidemiologic studies such as those of Epstein et al. should be expanded to cover other aspects of the habits and activities in addition to diet analyses of the racial groups involved. The high incidence of coronary artery disease in the Jewish group could not be attributed entirely to dietary factors.

The Epidemiology of Atherosclerosis Among a Random Sample of Clothing Workers of Different Ethnic Origins in New York City. I. Prevalence of Atherosclerosis and Some Associated Characteristics. F. H. Epstein, E. P. Boas, and R. Simpson. *J. Chronic Dis.* 5: 300, 1957.

The authors present a very interesting study of an attempt to study the etiology of atherosclerosis in a highly selected group of patients. The group studied was a "sample" from 33,000 men and women employed in the men's clothing industry in New York City. This group consisted mainly of first generation Jewish and Italian men and women. Dietary studies on this group have already been published in this journal. Included in the "Sample" were 683 men and 592 women.

The difficulty encountered in the study was expressed by the authors: "The importance of sampling and of using valid statistical methods of analysis has become recognized, but the clinical diagnosis of various manifestations of atherosclerosis has remained a difficult and unsatisfactory task."

The report includes information on relative weight, height, other bodily characteristics, blood pressure, serum lipid levels, prevalence of diabetes, and the general state of health of the "sample" group.

The study provides an example of a comprehensive epidemiologic study of manifest atherosclerosis and should establish a basis for comparison with other much-needed surveys of this kind. K. R. CRISPELL

The Epidemiology of Atherosclerosis Among a Random Sample of Clothing Workers of Different Ethnic Origins in New York City. II. Associations Between Manifest Atherosclerosis, Serum Lipid Levels, Blood Pressure, Overweight, and Some Other Variables. F. H. Epstein, R. Simpson, E. P. Boas. *J. Chronic Dis.* 5: 329, 1957.

This paper deals with the association between manifest atherosclerosis, serum lipid levels, blood pressure, overweight, and other variables, in the group of patients described in the previous abstract.

As reported previously by the authors in the JOURNAL the calorie intake and total fat content consumption among these Italian and Jewish populations were similar. However, the Italians used 32 per cent of

vegetable fat in their diet while this figure was only 20 per cent in the Jewish group. It is interesting, therefore, that the incidence of coronary artery disease was definitely increased among Jewish men. The authors believe that this increased incidence is unexplained, which is probably correct, but it seems conceivable that the increased animal fat used by the Jewish race may be a factor.

The authors feel that serum lipid levels, hypertension, obesity, and diabetes mellitus do not account for the demonstrated ethnic difference as they were similar in the two groups. The two groups were also well matched as to socio-economic status and the type of work done. This is an important study and should be continued for several more years. K. R. CRISPELL

Observations on the Variability of Total Serum Cholesterol in Johns Hopkins Medical Students. C. B. Thomas, and F. F. Eisenberg. *J. Chronic Dis.* 6: 1, 1957.

This is a report of observations on the variability of total serum cholesterol in Johns Hopkins' medical students. Cholesterol determinations were obtained from 759 students between October, 1948, and June, 1956.

The values varied from 108 to 407 mg/100 ml with a mean value of 228.7 mg/100 ml. The authors were able to verify that the level of cholesterol did increase with age, but that this was by no means the rule. There was considerable difference in the degree of cholesterol stability; in some subjects the levels were stable and in others labile on repeated occasions.

In these healthy subjects under 35 years of age, the variability in cholesterol levels was not explained by the slight difference in age of this group. No explanation is offered by the authors at this time.

This is an excellent study and is part of a broad investigation of possible precursors of hypertension and coronary artery disease. Such a study will be exceedingly valuable in settling the question of the relationship of cholesterol to these diseases. K. R. CRISPELL

Many studies on the effect of diet or dietary supplements upon serum lipids, especially cholesterol, have been published. These investigations are based on the supposition that the elevation of lipid correlates with the severity of atherosclerosis and that a reduction of the lipid level favorably affects the lesion. These points are open to question.

Diet and Coronary Heart Disease. Special Article *Chem. and Eng. News* 35, 24: 16, 1957.

This is an excellent up-to-the-minute review of the various theories thus far proposed and now being investigated relating to the etiology of coronary heart disease. The reviewer believes that "almost nothing has been proved beyond possible doubt. Many basic points are still merely good working hypotheses." Discussed pro and con are the following factors that have been proposed in relation to the subject: fats—saturated, unsaturated and hydrogenated; essential fatty acids and *trans* isomers; level of fat in the diet; other dietary factors such as methionine, choline, vitamin B_6 trace metals, proteins and carbohydrates; heredity; sex; obesity; exercise; stress and hypertension. The use of chemical remedies and anticlotting agents is discussed. The question is raised as to whether actually the percentage of fat in the American diet is greater now than it was 50 years ago. It is questioned also whether statistics dating back even 20 years were sufficiently accurate to justify the conclusion that there is an increasing prevalance of coronary heart disease. The U. S. Public Health Service is spending $24,000,000 a year on heart research. Other organizations have allocated similarly large amounts. "Another ten years may pass before the real answers are known about the relation of diet and coronary artery disease."

F. E. RICE

Calories and Cholesterol. A. Keys. *Geratrics.* 12: 301, 1957.

This is another in a series of very challenging papers by Dr. Keys. "The thesis for consideration here is that the diet influences both atherogenesis and thrombogenesis and is an important part of the reason for the differences between populations in the age-specific frequency of coronary heart disease." Much of the data in the paper has been presented previously, but this is a nice review of the author's present views on this complicated subject.

The great differences in the incidence of atherosclerosis between populations are not explained by race, nationality, climate, or any non-dietary factor so far studied. The differences in the frequency of coronary artery disease appear to be closely correlated with the average serum cholesterol concentration in samples of the populations. The differences are accounted for in the beta-lipoprotein fraction of the serum. It appears to be related to the proportion of fats in the diet and unrelated to the amount of protein. Unsaturated and linoleic acid fats affect the serum cholesterol level in the opposite direction to the saturated fats.

The author feels that hypercoaguability of the blood can be demonstrated after a fat meal. This may be due to kephalins rather than to fatty acids or neutral fats. The addition of corn oil to the American diet has been advocated as a method of reducing serum cholesterol levels. Keys believes that very large amounts would have to be used, and "this would mean an impossibly high calories intake."

In the author's words, "I doubt that the day will come soon when we can load our blood with fat without affecting cholesterol and blood coaguability. I see no prospect of preventing coronary heart disease merely by taking pills or capsules or periodic swallowing of corn oil." This is an article well worth reading in detail for those interested in this unsolved problem of atherogenesis. K. R. CRISPELL

A palatable diet effective in lowering serum cholesterol levels is claimed in the following paper. While low-fat diets are capable of maintaining a low lipid level over a period of two to six years according to one study (Roen, P. B.: Geriatrics 11: 200, 1956), they are extremely difficult to impose upon most patients.

A Diet Restricted in Refined Cereals and Saturated Fats: Its Effect on the Serum-Lipid Level of Atherosclerotic Patients. E. Van Handel, H. Neumann and T. Bloem. *Lancet* 1: 245, 1957.

Although low-fat diets in general lower the serum lipid levels and have been used for this in atherosclerotic patients, the authors have found the diet unsatisfactory from the patients' viewpoint and report here the results of a different regime. Lard and other animal fats, white bread and refined flour products were forbidden. Up to 25 g of butter or margarine was allowed. Whole meal bread, liquid soybean oil, lean meat, milk, eggs and cheese were allowed *ad lib*. There was an obligatory daily supplement of 2 to 3 g of soy lecithin, 25 to 50 g of peanuts, 25 to 50 g of peas or beans and 25 to 50 g of whole soy flour.

In 16 patients with a serum cholesterol under 250 mg/100 ml the diet had no significant effect after 30 to 360 days. In 14 with initial levels of cholesterol above this (from 235 to 400, average 300), there was a steady decrease in serum cholesterol to an average of 228 mg (172 to 250), even where this had been previously unaffected by a low-fat diet. F. E. HYTTEN

Considerable disagreement exists in the interpretation of laboratory data dealing with flotation values, lipoprotein levels, and lipid ratios in serum of patients under study for atherosclerosis. The measurement of serum cholesterol, aside from being the simplest method, appears to provide an adequate broad classification as to the extent of atherosclerosis.

Serum Lipoproteins in Preclinical and in Manifest Ischemic Heart Disease. J. T. Doyle, L. S. DeLalla, W. H. Baker, A. S. Heslin, and R. K. Brown. *J. Chronic Dis.* 6: 33, 1957.

The authors report studies on serum lipoproteins in both preclinical and manifest ischemic heart disease. There were 76 men in the first group and 48 in the later group. The age spread was from 40 to 55 years. In the group with manifest heart disease, 28 had survived a myocardial infarction and 20 had angina pectoris.

The survivors of myocardial infarction as a group showed significant elevations in lipoprotein-cholesterol and phospholipid values. This was accounted for by an increase in the β-lipoprotein fractions. There was a wide overlap in all groups, so that individual values were not of diagnostic value. The flotation fractions were also determined. The authors conclude from their study that serum cholesterol values provide as much information as other more elaborate chemical procedures. K. R. CRISPELL

The Fatty Acids of the Blood in Coronary-Artery Disease. A. T. James, J. E. Lovelock, J. Webb, and W. R. Trotter. *Lancet* 1: 705, 1957.

In twelve patients with coronary artery disease, nine with infarcts and three with angina of effort, and 12 comparable controls free from any suggestion of coronary disease, blood samples were examined by gas-liquid chromatography to determine the range of fatty acids from C_6 to C_{20}. Three fractions were studied: red cells, plasma phospholipid and plasma acetone-soluble fractions.

There were no detectable differences and controls between patients in the proportions of the fatty acids in the red cell and phospholipid fractions. In all three fractions the mean proportions of the "essential fatty acids" (linoleic and arachidonic) were similar in the two groups.

In the acetone-soluble fraction of the plasma, "there was some suggestion of an increase" (this barely reached statistical significance for any component) in the combined proportions of mono-unsaturated C_{14}, C_{16} and C_{18} acids in the patients compared to the controls. "The implications of this observation cannot be properly assessed without further resolution of the acetone-soluble fraction into its two components, cholesterol esters and glycerides." F. E. HYTTEN

The accumulation of exogenous carotenoid pigments in atherosclerotic placques in constant ratio with the cholesterol content of the lesion favors the interpretation that lipids are deposited in the vessel wall from the plasma rather than formed locally by the tissue.

The Distribution of Epiphasic Carotenoids in Atherosclerotic Lesions. D. H. Blankenhorn, D. G. Freiman and H. C. Knowles, Jr., *J. Clin. Investigation* 35: 1243, 1956.

Carotenoid pigments are colored lipids distributed widely throughout nature. Certain carotenoids, chiefly those without hydroxyl groups, can be converted to vitamin A. Studies have indicated that carotenoids cannot be synthesized by man. It was thought advisable, therefore, to study the occurrence of these lipids in atheromatous plaques, as evidence that dietary lipid may be deposited in the lesions. The concentrations of total carotenoids and total cholesterol were studied in thirty human aortas with varying degrees of atherosclerosis.

With increase in the severity of the lesions, there were accompanying significant increases in carotenoid and cholesterol contents. The carotenoid-cholesterol ratio was relatively constant. Although cholesterol in the plaque may accumulate either inside the plaque or outside, it is generally accepted that carotenoids are derived from exogenous sources. Although it is not known whether carotenoid pigments are concerned with the pathogenesis of atherosclerotic lesions, the present findings give evidence that one dietary lipid is present

not only in atherosclerotic plaques but accumulates in direct proportion to age and extent of the lesions.

S. O. WAIFE

The use of phytosterols in reducing serum lipids in man has been limited by the large doses needed and the transient effects reported in some studies. Lipotropic agents are without effect upon serum cholesterol or anginal symptoms in man. Further studies on the compound reported below will be of interest in this regard.

The Hypocholesterolemic Effect of Phenylethylacetic Acid Amide in Hypercholesterolemic Atherosclerotic Patients. B. Rossi and V. Rulli. *Am. Heart J.* 53: 277, 1957.

Plasma cholesterol levels (Bloor's method) were studied in relation to administration of phenylethylacetic acid amide to 14 patients 39 to 70 years old, with clinical evidence of atherosclerosis and with initial plasma cholesterol levels above 200 mg per 100 ml on a low-fat diet (500 to 600 mg cholesterol and 20 to 30 mg neutral fat per day). Phenylethylacetic acid amide was given orally after meals, 2 g per day for two weeks and then 3 g per day for one to eight additional weeks. Plasma cholesterol was measured at weekly intervals and one month after the drug had been discontinued.

All patients except one showed a reduction of plasma cholesterol in the majority of determinations during the period of treatment. The ten patients treated for eight to ten weeks showed a mean reduction of plasma cholesterol of 43.3 per cent (range 5 to 71 per cent) at the end of this period. One month after discontinuing the drug the plasma cholesterol was only 6.8 per cent below the initial level. No clinical or toxic effects of the drug were evident.

W. H. ABELMANN

ATHEROSCLEROSIS: EXPERIMENTAL NUTRITIONAL STUDIES

Experimental animals of all varietis have been made susceptible to atherosclerosis. The vascular defects produced differ in certain histopathologic characteristics due probably to differences in species susceptibility and to variations in the dietary regimens employed. Several investigators have reported an inverse relationship between serum and liver cholesterol content and the level of dietary protein. The following abstracts demonstrate the differences in species responses to variations in protein intake, the rat being more resistant to atheroma formation and showing less change with protein restriction.

Effect of Low Protein Diet on the Serum Lipids and Atherosclerosis of Cholesterol-fed Chickens. E. A. Nikkila and O. Ollila. *Acta Path. Microbiol. Scand.* 40: 177, 1957.

The purpose of the investigation was to study the relation of the diet composition to cholesterol-induced atherosclerosis of chickens; effects of very low-protein diet are presented. One hundred chicks four weeks old were separated into four groups of twenty-five, each group on a different diet. One group received a 3.5 per cent protein diet, another group received the same diet plus 1.5 per cent cholesterol. There were two control groups, i.e. one on the usual 20 per cent protein diet, the other with the same diet plus 1.5 per cent cholesterol. Ninety-six chickens survived a 25-week experimental period and were sacrificed for blood analysis of total cholesterol, phospholipids, and proteins. Aortae were studied histologically and atheromatous lesions graded; other organs were also studied.

The low-protein diet alone did not raise total serum cholesterol significantly, but the low-protein diet plus 1.5 per cent cholesterol did produce markedly elevated total serum cholesterol levels. Aortic atheromatosis was also marked. The fact that the low-protein diet without cholesterol supplement did not produce a hypercholesteremia is considered to be due to simultaneous depression of cholesterol synthesis and removal. Lowered phospholipid concentration in chickens on the low-protein diet was interpreted as an indication of the decreased capacity of the liver to synthesize phospholipids. Cholesterol-fed animals on a low-protein diet showed evidence of bilirubin retention said to be a destructive effect of dietary cholesterol overload. Future experiments should be designed to explain specifically the nature of those deficient factors which enabled the cholesterol-overload effect to occur.

E. COHEN

Certain Dietary Effects on the Serum Cholesterol and Atherogenesis in the Rat. R. J. Jones, R. W. Wissler, and S. Huffman. *A. M. A. Arch. Pathol.* 63: 593, 1957.

The effect of various diets on the evolution of coronary arteriosclerosis was studied in young Sprague-Dawley rats 12-weeks old at the beginning of the experiment. The composition of the diets varied in their content of lard, dextrin, casein, choline and cholesterol. Moreover, some groups received supplements of 0.5 per cent DL-methionine or 0.02 per cent alpha-tocopherol for six months alone or combined up to 12 months. Total lipids, cholesterol, lipid-phospholipids and their ratio were determined; changes in vessels, myocardium, skeletal muscle and testes were examined microscopically. Coronary sclerosis developed in rats fed a diet with only casein, as a source of protein and lard as a source of fat. The incidence of the lesions did not correlate with the level of fat or choline in the diet. The incidence was not changed by decreasing the level of protein or adding a supplement of hypercholesteremic substances. The lesions were not reversed or prevented by alpha tocopherol alone nor by methionine alone, the latter accentuated hypercholesteremia. Hypercholesteremia was, however, reduced to normal values, when both methionine and α tocopherol were added to the diet.

M. SILBERBERG

Experimental atherosclerosis was studied first in the rabbit. While nutritional correlations with the human

disease can be made, there are many features which are lacking such as the thrombotic-occlusive component.

Severity of Atherosclerosis in Rabbits in Relation to Serum Lipids and to Aorta Cholesterol Content. A. J. Day and G. K. Wilkinson. *Australian J. Exper. Biol. & M. Sc.* 34: 423, 1956.

Although this subject has been extensively studied, the authors reinvestigated the relationship of cholesterol levels with the severity of atheroma in cholesterol-fed rabbits. It was found that a close correlation exists between the severity of the aortic atheroma and the serum cholesterol and β-lipoprotein levels. On the other hand, no consistent correlation was found to exist between phospholipids, fatty acids, and cholesterol phospholipid (C/P) ratio and severity of atheroma. There was also no correlation between the α-lipoproteins and atheroma. S. O. WAIFE

The Effect of Uranium Acetate on Serum Lipids and on Atherosclerosis in Cholesterol Fed Rabbits. A. J. Day, C. J. Schwartz, G. K. Wilkinson, and J. A. Peters. *Australian J. Exper. Biol. & M. Sc.* 35: 31, 1957.

Uranium acetate administration in cholesterol-fed rabbits produces a highly significant increase in the rate of rise of serum phospholipid and serum fatty acids, but no significant change in the rate of rise of serum cholesterol. The uranium acetate administration did not cause any increase in the atherosclerosis resulting from the cholesterol feeding. AUTHORS

Sitosterols have been employed to lower serum cholesterol le . . by interference with its intestinal absorption. However, work published recently suggests that plant sterol-esters may be absorbed and converted to cholesterol or cholesterol intermediates capable of inducing atherosclerosis.

The Effect of Beta-Sitesterol on Cholesterol-Induced Atheroma in Rabbits with High Blood Pressure. R. H. Heptinstall and K. A. Porter. *J. Exp. Pathol.* 38: 49, 1957.

New Zealand and Chinchilla male rabbits weighing 2.5 kg were made hypertensive by putting a clip on the left renal artery two weeks subsequent to right-side nephrectomy. One group of these animals received 0.75 g cholesterol/100 g. The total intake of cholesterol varied with the individual from 3.5 to 4.2 g/weeks. The second group was given the same diet as the first group supplemented by β-sitosterol in amounts three times that of cholesterol. Water was available at all times. Weights were taken regularly, blood serum cholesterol was determined once and blood pressure twice a week. The animals were sacrificed after eight weeks. Arcus senilis had developed in all but one of the animals fed cholesterol, but in none of the animals fed the supplement of β-sitosterol. At necropsy, the atheromas were graded planimetrically according to extension and elevation of the plaques. The atheromas were considerably smaller in the animals treated with β-sitosterol in spite of the fact that fluctuations and rise in blood pressure were about the same in both experimental groups. Throughout the period of observation, the total cholesterol serum values and the adrenal weight carcass ratio were likewise lower after treatment with β-sitosterol. For the cholesterol-fed group serum cholesterol values showed a mean of 1104 ± 318 mg/100 ml and the adrenals a mean weight of 0.068 ± 0.015 g as compared to mean cholesterol values, of 315 ± 154 mg/100 ml and adrenal weights of 0.043 ± 0.014 g for the rabbits receiving β-sitosterol. Serum lipoidosis was rare. However, cholesterol infiltrations we marked in liver, spleen and adrenals of the animals fed sitosterol. M. SILBERBERG

Corn oil, a source of essential fatty acids, has been employed as a means of lowering serum cholesterol levels. The mechanism of action of the unsaturated fatty acids is not understood and is the subject of intensive investigation.

Saturated Versus Unsaturated Fats in Experimental Arteriosclerosis. W. A. Thomas, N. Konikov, R. M. O'Neal and K. T. Lee. *A. M. A. Arch. Pathol.* 63: 571, 1957.

The effect of corn oil, a relatively unsaturated fat, on the development of induced thromboembolic pulmonary arteriosclerosis was compared with that produced by saturated fats such as butter and oleomargarine. Male and female young adult New Zealand white rabbits kept on a standard diet of Purina rabbit pellets were given by stomach tube (a) 25 ml of warm corn oil; (b) butter fat; and (c) isocaloric amounts of sucrose in water. All animals were injected with blood clots obtained from 10 ml of blood once a week for six weeks. The changes in the pulmonary vessels were studied microscopically and graded according to severity. In contrast to findings with highly saturated fats, feeding of corn oil had no demonstrable effect on the development of arteriosclerosis. This difference may be due to the degree of saturation of the various fats or to the content of essential fatty acids. M. SILBERBERG

Swine and Experimental Atherosclerosis. J. H. Bragdon, J. H. Zeller, and J. W. Stevenson. *Proc. Soc. Exp. Biol. & Med.*, 95: 282, 1957.

Eight two-year-old boars were fed a basal diet enriched with 22 per cent corn oil or 26 per cent butter for periods of nine weeks. Animals fed the ration containing butter showed an increase in high density alpha-lipoproteins, an effect not noted in animals given isocaloric amounts of corn oil. At autopsy 50 per cent of the animals fed butter showed atheroma-like lesions in the aorta characterized grossly by thickening of the intima of the descending thoracic aorta, an incidence which was also observed in animals fed conventional stock rations. Microscopically the aortic lesions contained cells of the histiocytic type, showing in their

cytoplasm varying quantities of sudanophilic material and round cells, their cytoplasm filled with unidentified homogeneous non-staining material. These lesions thus resembled those seen in some types of human atherosclerosis. M. SILBERBERG

Gallogen is a camphoric acid ester which has been demonstrated to have a choleretic action. Whether or not this effect is responsible for its hypocholesterolemic action is a matter for further study.

Effect on Experimental Avian Atherosclerosis of Dietary Oils and Gallogen. J. S. King Jr., T. H. Clarkson, and N. Hyder Warnock. *Proc. Soc. Exp. Biol. Med.* 93: 443, 1956.

Young cockerels were fed Purina Growing Mash and 1 per cent cholesterol with a supplement of 8 per cent peanut oil, 8 per cent coconut oil or 8 per cent sardine oil respectively, alone or in combination with 0.1 per cent Gallogen (the diethanolamine salt of the monocamphoric acid ester of α,4-dimethylbenzyl alcohol). The addition of Gallogen to the various oils decreased the rise of cholesterol in aorta and liver but only slightly in the serum. Gallogen was most effective if the unsaturated sardine oil was used as a dietary supplement, it was less effective after use of the moderately unsaturated peanut oil and least after use of the saturated coconut oil. M. SILBERBERG

Sulfated compounds with heparin-like antilipemic action have been under study for several years. The ability of sulfated alginic acid to lower serum cholesterol and prevent deposition of this lipid in atheromata is of considerable interest.

Effects of Sulfated Polysaccharides on Pre-established Atherosclerosis. P. Constantinides, P. Saunders, and A. Wood. *A. M. A. Arch. Pathol.* 62: 369, 1956.

Ninety-six white New Zealand rabbits were fed a diet containing 1 per cent cholesterol for three months. Subsequently, they were separated into four groups which were (1) killed immediately; (2) continued on the cholesterol diet for an additional two months; (3) fed the cholesterol diet and injected daily with from 5 mg/-kg to 7.5 mg/kg heparin; (4) fed the cholesterol diet and injected with sulfated alginic acid (SAA), an antilipemic agent, in doses ranging from 3.5 mg/kg to 5 mg/kg. The SAA was more antilipemic than heparin; it lowered the hypercholesteromia, while heparin did not do so. Both SAA and heparin caused lipid depletion of pre-established aortic atheromas, but SAA prevented further deposits of cholesterol in the aortic atheromas. Both substances inhibited formation of xanthomatous deposits in the kidney. Heparin suppressed, but SAA promoted the appearance of foam cells in spleen, mesenteric lymphnodes and renal glomeruli. This may be due to the lipolytic action of SAA. M. SILBERBERG

Any factor, whether mechanical, metabolic or chemical, which injures or stresses a vascular bed may produce alterations within the wall favoring the deposition of cholesterol and true atheromatous changes.

The Effect of a Brief Period of High Blood Pressure on Cholesterol-Induced Atheroma in Rabbits. R. H. Heptinstall and K. A. Porter. *J. Exp. Pathol.* 38: 55, 1957.

New Zealand and Chinchilla male rabbits were divided into three groups. In the first series the right kidney was removed, and after two weeks a clip was put on the left renal artery. Throughout the experimental period of eight weeks the rabbits received cholesterol with a total intake of 3.5 to 4.2 g/week. In the second series, the arrangement of the experiment was the same as in the first except that the clip on the renal artery was taken off after 14 to 21 days. In the third series, the animals were treated as in the second series except that cholesterol was fed only after removal of the clip. The atheroma formed were measured planimetrically, and blood pressure, blood serum cholesterol and adrenal carcass weights were determined. In all groups, stabile cholesterol levels were established after four weeks. In the first group, the blood pressure was high throughout the period of observation; in the second group it was elevated for two to three weeks, and thereafter it returned to normal; in the third group, the blood pressure was normal or at the most slightly elevated. Atheroma formation was most marked, and blood serum cholesterol, and adrenal weights were highest in animals of the first group, next in line were those of the second group, followed by those of the third group. Thus, the degree of atheroma depended upon the duration and the degree of the hypertensive state. M. SILBERBERG

NUTRITION AND THYROID

The thyroid gland rapidly accumulates and oxidizes iodide, which is incorporated iuto amino acids (tyrosine and histidine), and in the storage protein, thyroglobulin. The active hormones derived from the enzymatic proteolysis of this protein are thyroxine and 3:5:3'-triiodothyronine. At the cellular level, additional transformations occur to produce their acetic acid analogues. It is probable that various metabolic effects are achieved by these individual hormones rather than a single hormone. The effect of the thyroid hormones upon lipid metabolism is of particular interest as possible preventative agents in the treatment of atherosclerosis.

Long-Term Effect of Dried Thyroid on Serum-Lipoprotein and Serum-Cholesterol Levels. B. Strisower, J. W. Gofman, E. F. Galioni, J. H. Rubinger, J. Pouteau, and P. Guzvich. *Lancet* 1: 120, 1957.

It has been shown previously from this laboratory that serum levels of cholesterol and of low-density lipoproteins fell on a dosage of 195 mg daily of dried thyroid

substance, but that an "escape" occurred—the levels rising again to normal in spite of continued treatment. It was suggested that this "escape" might be due to the suppression of endogenous thyroid hormone, and that a larger dose of the dried substance might have been more continuously effective.

Thirty-nine schizophrenic but "physically and metabolically normal" persons were the subjects of the present investigation. They received 195 mg daily of dried thyroid for 30 weeks, followed by 260 mg for 39 weeks and 325 mg for 36 weeks. The two higher dosages, but not the lowest, produced a sustained fall in serum levels of both cholesterol and the low density lipoproteins and no "escape" occurred.

It is suggested that "dried thyroid is worthy of consideration as a prophylactic agent against coronary heart disease." F. E. HYTTEN

The Influence of Triiodothyroacetic Acid on the Circulating Lipids and Lipoproteins in Euthyroid Men with Coronary Disease. M. F. Oliver, and G. S. Boyd. *Lancet* 1: 124, 1957.

Twelve hypercholesterolemic men between the ages of 33 and 49 who had experienced a proved myocardial infarction more than nine months previously were selected as experimental subjects.

To 6 of the men "triac" (triiodothyroacetic acid) was given orally in increasing doses from 0.5 mg to 4.0 mg daily. No effect was noticed until a dose of 3.0 mg daily was reached and with 4.0 mg for ten days the mean plasma cholesterol had fallen by 21 per cent. The mean B.M.R. was not raised but two men had rises of 13 and 20 per cent and two experienced angina of effort which disappeared when treatment stopped.

The six other men had daily doses of 3 rising to 5 mg for three months. There were significant falls in the plasma cholesterol, cholesterol/phospholipid ratio, and the β-lipoprotein-cholesterol. The mean B.M.R. was not raised: one man had a diminished exercise tolerance and increased angina of effort. The fall in plasma cholesterol could not be maintained for long even by increasing the dosage of triac.

It is concluded that this drug is unsuitable for the long term control of hypercholesterolemia in patients with clinical coronary disease. F. E. HYTTEN

Effect of Triac on the Reabsorption of Cholesterol from Atheromatous Lesions in the Aorta of the Rabbit. R. Pitt-Rivers and W. R. Trotter. *J. Exp. Pathol.* 38: 97, 1957.

Rabbits were fed a stock diet supplemented by 1 g of cholesterol daily for 12 weeks. After discontinuation of the cholesterol feeding, one half of the animals were given daily per os for 26 weeks 200 to 300 μg of triiodothyroacetic acid in 10 per cent sucrose. The serum cholesterol levels were plotted against the cholesterol levels of the atheromatous lesions that had developed in the aorta and compared with the values obtained in the other half of the control rabbits that had not received triac. Triac failed to change materially the size or the cholesterol content of the atheromatous lesions in the aorta as compared with the rabbits not treated with triac. M. SILBERBERG

The metabolic effects of thyroxine appear to require a "permissive" level of adrenal steroids in order to be fully active. In the absence of adrenocortical secretion, thyroid administration may produce an Addisonian crisis.

Hormonal Factors Influencing Calorigenesis. E. S. Evans, A. N. Contopoulas, and M. E. Simpson. *Endocrinology* 30: 403, 1957.

This study was conducted because certain observations have indicated that pituitary factors other than thyrotrophic hormone (TSH) may produce an elevation of the metabolic rate (MR). This investigation was carried out by administering various pituitary hormones to rats that had been hypophysectomized, thyroidectomized, or both hypophysectomized and thyroidectomized.

It was found that TSH elevated the MR of hypophysectomized rats although normal values were not obtained. TSH had no influence on MR of thyroidectomized animals. Adrenocorticotrophic hormone (ACTH) increased the MR of rats hypophysectomized, thyroidectomized, or both hypophysectomized and thyroidectomized. Although ACTH did produce elevated metabolic rates in thyroidectomized rats, the increase was not sufficient to restore the MR to normal. It was shown that the caloric action of ACTH was dependent upon the presence of intact adrenal glands and also that hydrocortisone and cortisone administered to thyroidectomized rats raised the MR to normal. A synergistic effect of both TSH and thyroxine with hydrocortisone was also demonstrated. Neither the melanocyte stimulating hormone or the lactogenic hormone had an effect on the low MR of the hypophysectomized animal.

The authors believe that thyroid and adrenocortical hormones act conjointly to control the MR but either can act independently to influence the level of calorigenesis. A. B. EISENSTEIN

Factors Influencing Survival of Rats in Fasting Metabolic Rate and Body Weight Loss. R. H. Rixon and J. A. F. Stevenson. *Am. J. Physiol.* 188: 332, 1957.

The individual duration of survival of adult rats in complete fasting varied considerably; the range at an environmental temperature of 22° C was 6 to 16 days, at 2 to 5° C, 1 to 7 days, and in thyroidectomized animals at 22° C, 15 to 25 days. This variation in survival was not closely related to the initial body weight but was related to the individual proportionate body weight loss per day and the total proportionate weight loss sustained before death. The individual proportionate rate of weight loss has been correlated with the metabolic rate indicating that the former reflected the met-

abolic rate of the animal. The duration of survival in fasting has been correlated with the individual metabolic rate, whether measured before or during fasting. Since fasting did not obliterate or reduce the individual differences in metabolic rate, it was possible to predict the individual duration of survival from knowledge of the prefasting metabolic rate. The total proportionate weight loss, which also influenced the survival time in fasting, was altered by changes in the environmental temperature and probably by other factors. The previous diet whether high in protein, fat, or carbohydrate had little effect on the duration of survival. Fasting caused a decrease in the metabolic rate of intact rats at 22° C but no change in that of thyroidectomized rats or of rats living in the cold. AUTHORS

The principal hypotheses offered to explain the inhibitory effect of iodide upon the thyroid are (a) inhibition of release of thyrotropin from pituitary, (b) direct inactivation of circulating thyrotropin, and (c) inhibition of hormone production and release by thyroidal cells. The data presented favors the third postulation. Excessive iodide administration may inhibit the thyroid gland sufficiently to produce myxedema.

The Effect of Stable Iodide on Thyroid Secretion in Man. M. A. Greer and L. J. DeGroot. *Metabolism* 5: 682, 1956.

The thyroid secretion rate was determined in 15 patients with thyrotoxicosis, 16 euthyroid patients, and in one with hypothyroidism. A tracer dose of I^{131} was given orally, 24 hours after which methimazole was administered to block I^{131} reaccumulation. The secretion rate was determined as the half-time in days of the decrease in radioactivity over the gland. Sodium iodide was given during the experimental period in order to observe its effect upon the secretion rate. In patients with thyrotoxicosis, sodium iodide produced a marked slowing of thyroidal secretion. In six of the eight thyrotoxic patients given moderate doses of thyrotropin the secretion rate of thyroid hormone returned to its initial slope despite the continued administration of iodide. In four thyrotoxic patients, the inhibitory effect of iodide was related to the serum iodide levels. No effect upon endogenous secretion rates in nonthyrotoxic patients was noted upon administration of sodium iodide. However, when TSH was given to euthyroid patients, iodide induced a slowing of thyroidal secretion similar to that observed in thyrotoxicosis. In one instance, increasing the dose of TSH during iodide inhibition resulted in an increase in the secretion rate. Thiocyanate had no effect upon iodide inhibition in three thyrotoxic and three euthyroid patients. It is suggested that TSH and iodide have a mutually antagonistic action on some intrathyroidal mechanisms responsible for the release of thyroid hormone. In addition, iodide may act to inhibit the release of thyrotropin from the pituitary. C. R. SHUMAN

Myxedema and Goiter Attributed to Iodine Ingestion in a Patient Subsequently Developing Hyperthyroidism. V. P. Vanderloon. *Metabolism* 5: 640, 1956.

A patient under treatment for asthma with a mixture of salts providing 2 g of iodine daily developed frank myxedema with diffuse goiter formation over a period of three years. One year after discontinuance of iodine, classical manifestations of thyrotoxicosis developed. It was noted that a strong family history of thyroid disease was present on the maternal side. The mechanism by which iodine acts as an antithyroid agent has not been clarified. The liability to myxedema from iodine treatment may be an evidence of an intrinsic deficiency in self-regulatory mechanisms of the thyroid gland in a patient predisposed to hyperthyroidism.

C. R. SHUMAN

Myxedema Induced by Prolonged Iodide Administration. H. M. Rubinstein and L. Oliver. *New England J. Med.* 256: 47, 1957.

Recently it has been shown that prolonged administration of large amounts of iodide for treatment of pulmonary conditions in normal people could result in goiter and hypothyroidism. In this paper a case of this type is described.

For a period of six years a female patient with bronchial asthma had been taking without presciption 244 mg of iodide daily. A month before admission to the hospital she raised her consumption to 1,784 mg daily. Clinical examination showed myxedema, a diffuse swelling of the thyroid to twice its normal size and a basal rate about 20 per cent below normal. Radioiodine was accumulated rapidly by the thyroid and then was discharged rapidly suggesting that the thyroid iodide-trapping mechanism was working normally but that the iodide was not being converted to organically-bound iodine. A week after treatment all the administered radioiodine was taken up by the thyroid and was not discharged even after administration of potassium thiocyanate, indicating that all the radioactive iodine was organically bound. The very high secretion rate of protein-bound iodine indicated a hyperthyroid condition. Unfortunately there were no later tests with radioiodine to determine whether or not the patient became euthyroid. Other clinical observations indicated, however, that the patient became euthyroid six to eight weeks after treatment started. The authors believe that the high serum iodide level, in some way, interferes with the synthesis of the thyroid hormone. M. W. BATES

The anemia of myxedema has been recognized for many years. This is usually normocytic and normochromic and responds promptly to treatment with desiccated thyroid. Some of the iron dynamics have been studied in thyroidectomized animals in the following paper.

Thyroid and Iron Metabolism. M. E. Anstoni, D. Ziliotto, and E. Odeblad. *Acta. Med. Scandinav.* 155: 329, 1956.

The utilization of iron by bone-marrow and erythropoietic tissues of thyroidectomized rats was investigated using scintillation counting and autoradiography following radioactive iron administration. The bone marrow of the thyroidectomized animals was found to take up less Fe^{59} at a slower rate than that of control animals. Similar data were recorded for the red blood cells. The spleens and livers of the thyroidectomized animals appeared to take up larger amounts of iron than in the control animals. The iron contents of the stomach, intestines, and kidneys indicate that the tendency to preserve iron is retained despite the iron availability in deposit organs. It appears that thyroidectomy has a definite influence on iron metabolism and may produce a hematopoietic disorder. The thyroid hormone apparently plays a role in the regulation of iron utilization in the organism. C. R. SHUMAN

The enhanced peripheral utilization of thyroxine postoperatively in normal patients corresponds to the higher rate of nitrogen loss and elevated adrenocortical activity after operation in this group. In debilitated patients, peripheral thyroxine utilization is reduced as are nitrogen losses and adrenal steroid levels.

The Effect of Operative Trauma on the Utilization of Thyroid Hormone. I. S. Goldenberg, P. J. Rosenbaum, C. White and M. A. Hayes. *Surg. Gynec. & Obst.* 104: 295, 1957.

In a series of previous articles, Goldenberg and his associates have presented data suggesting an apparent abrupt, transient increase in activity of the thyroid gland after operation, particularly in the acutely ill (as opposed to the chronically ill) patient. These data were an increased uptake of radioiodine by the thyroid and an increase in circulating protein-bound radioiodine in the very early postoperative period. In the present paper, the rate of "disappearance" of labeled thyroxine from the plasma (measured as the changing conversion ratio (net counts PBI^{131} in 2 ml serum/net counts in 2 ml serum)) were compared pre- and postoperatively as an index of thyroxine utilization. The control observations were made during the week preceding operation; the postoperative observations were made starting at operation and continuing for six days. Endogenous thyroid hormone production was blocked by administration of unlabeled iodine, orally or intravenously.

Eight patients, 18 to 78 years old, were studied before and after major operations under general anesthesia (specific agents used not mentioned). In the four patients who had either not experienced acute stress in the recent preoperative period or long standing chronic illness, the rate of decline of the conversion ratio in the plasma was faster after operation. In contrast, the other four patients had either severe acute stress or advanced chronic illness prior to operation; in these, the decline of the conversion ratio in the plasma was not changed by operation. S. M. LEVENSON

The influence of thyroid hormones upon bone, dental structures, and salivary glands are reported in the following papers.

The Influence of Dietary Thyroid on the Bones and Periodontium of Rats on Total and Partial Tryptophan Deficiencies. L. A. Bavetta, S. Bernick, and B. Ershoff. *J. Dent. Res.* 36: 13, 1957.

Tryptophan deficiency was produced in rats by a diet in which the other amino acids were supplied by acid-hydrolyzed casein. Apart from the amino acid source, it was a diet of good nutritional characteristics. Three other groups of rats were fed the same diet supplemented with 0.6, 1.2, or 2.4 g of DL-tryptophan per kg of diet. Another group was fed the same diet except that the acid-hydrolyzed casein was replaced by untreated casein with no tryptophan supplied. Rats on the tryptophan-deficient diet lost weight throughout the four-week experimental period. Those on the highest tryptophan supplement grew reasonably well, but were quickly outdistanced by the controls on the untreated casein diet. The rats on the other two levels of tryptophan barely maintained their original weight, or gained slowly. Other comparable tryptophan-deficient and tryptophan-supplemented groups were maintained with 0.5 per cent U.S.P. desiccated thyroid in the diet to produce a moderate hyperthyroidism. Growth of rats on each of the thyroid-supplemented rations was appreciably less than for those in the comparable normal group. The totally deficient rats died before the termination of the four-week period.

Diets containing 0.6 g of tryptophan per kg of diet or less, caused a marked inhibition and retardation of both endochondral and periosteal bone formation. Marked osteoporosis was observed in the alveolar bone. There is no reason to believe that these abnormalities had unique characteristics that would be characteristic of tryptophan deficiency. At the higher levels of tryptophan supplementation, the above abnormalities were reduced to minimal levels. When hyperthyroidism was produced, the bone pathology was materially augmented in the deficient rats and those fed low levels of tryptophan. However, 0.24 per cent of tryptophan was capable of preventing bone pathology, in the presence of hyperthyroidism. Ulceration of gingival epithelium with the formation of craters occurred in rats fed thyroid supplements to a tryptophan-free diet supplemented with 600 mg or less of tryptophan per kg. No comparable findings were observed in the absence of desiccated thyroid.

The authors attributed the poorer growth and more severe bone lesions among hyperthyroid rats to an increase in tryptophan requirement. While the latter probably occurred, more than likely the overall nutritional requirements of the rat were increased in a variety of ways to further penalize the hyperthyroid rats. It would be interesting to know to what extent this effect would occur in rats where the diet was only tryptophan-deficient with the tryptophan controls growing at rates

comparable to those on the supplemented diet with the untreated casein. J. H. SHAW

The Effect of Desiccated Thyroid, Propylthiouracil, Testosterone, and Fluorine on the Submaxillary Glands of the Rat. W. G. Shafer, and J. C. Muhler. *J. Dent. Res.* 35: 922, 1956.

Supplementation of the albino rat with 10 to 60 mg of desiccated thyroid per day in the diet resulted in a marked increase in the size of the tubules of the submaxillary gland with the accumulation of large numbers of granules within the cells. Supplements of propylthiouracil in the diet in a concentration of 100 mg/100 g of food caused a pronounced decrease in the size of the tubules with a striking reduction in the number of granules amounting almost to complete granule disappearance. Ingestion of 20 ppm of fluoride as sodium fluoride in the drinking water had no influence on the histologic appearance of the glandular tissue. Simultaneous administration of fluoride with desiccated thyroid or of fluoride with propylthiouracil did not alter the histologic picture caused by the desiccated thyroid or the propylthiouracil alone. Testosterone administration to male rats caused a slight but statistically insignificant enlargement of the tubules; in females, a significant increase in size was noted. Simultaneous administration of testosterone and of desiccated thyroid caused greater increases in tubular size than did either separately.

The question naturally arises as to whether these histologic changes in the submaxillary gland are indicative of functional changes in these glands. No comparable histologic changes were observed in the sublingual and parotid glands. J. H. SHAW

THE NERVOUS SYSTEM AND NUTRITION

In a few instances, pellagrins have been shown to be suffering with a familial disease known as Hartnup syndrome. The underlying cause of this condition may be an interruption of the reactions proceeding from tryptophan to niacinamide. The important features of this disorder are found in a report by Baron.

Hereditary Pellagra-like Skin Rash with Temporary Cerebellar Ataxia, Constant Renal Amino-aciduria, and other Bizarre Biochemical Features. D. N. Baron, C. E. Dent, H. Harris, E. W. Hart, and J. B. Jepson. *Lancet* 2: 421, 1956.

A new syndrome (H disease) is described in great detail as it affects four of eight children (aged 6 to 19 years) of a first-cousin marriage.

The most common clinical feature is a tendency to develop a rough reddened dermatosis in sunlight; the rash may be severe and appears identical with pellagra. On occasion a severe temporary cerebellar ataxia develops. Three of the children are mentally retarded.

The most specific abnormality associated with the syndrome is a constant and gross renal aminoaciduria of unique pattern; there is no evidence of other renal dysfunction. Indole-3-acetic acid and indican are also excreted in the urine in large amounts. The feces contain a moderately increased quantity of protoporphyrin. It is suggested that the immediate biochemical disorder producing the clinical syndrome is an abnormality of nicotinic-acid utilization. F. E. HYTTEN

Neurologic syndromes have been recognized in deficiency states involving pyridoxine and pantothenic acid within recent years. The symptoms attributed to gluten sensitivity may be related to a conditioned deficiency state involving one of these or other vitamins.

Bread and Tears—Naughtiness, Depression and Fits Due to Wheat Sensitivity. G. Daynes. *Proc. Roy. Soc. Med.* 49: 391, 1956.

A syndrome of allergy to wheat flour is described. This is self-limiting and seems to follow an acute infection; it has been called "the preceliac syndrome."

In children it is characterized by behavioral changes. The child becomes naughty, irritable and spiteful, passes pale bulky stools, sleeps poorly with outbursts of screaming, has poor appetite and weight loss. In some there may be *petit mal*-like attacks and skin rashes. The condition responds immediately to a gluten-free diet and recurs when wheat flour is re-introduced. In adults symptoms are similar but headache, insomnia, and depression are outstanding. The syndrome shows many similarities to canine hysteria which has been linked to agenized wheat flour. The author has observed that the severest attacks are precipitated by stale wheat flour. F. E. HYTTEN

Effect of Dietary Factors on Incidence of "Spontaneous" and Induced Convulsions in C3H Male Mice. Y. Ch. P. Lee, O. Jardetzky, J. T. King, and M. B. Visscher. *Proc. Soc. Exp. Biol. Med.* 95: 204, 1957.

Spontaneous convulsions had been observed in single-housed mice of strain C3H, especially after handling. These convulsive seizures appeared from six months of age on and varied with the diet given. The relationship between spontaneous and induced convulsions to dietary factors is analyzed in the present paper. In one experiment in which the animals were permitted to live to the end of their natural life span, 220 male mice of strain C3H were used. The animals were divided into groups of twenty each and were placed on 11 different dietary regimens including a basal diet containing 20 per cent fat; commercial stock diet usually contains about 5 per cent fat. In a second experiment six groups of 20 mice each were placed on diets containing, respectively, 17 per cent lard, 17 per cent lard + 30 mg/100 ml α tocopherol, and 5 per cent lard, 17 per cent hydrogenated vegetable fat, 17 per cent hydrogenated vegetable fat + 30 mg 100 ml α-tocopherol, and 5 per cent hydrogenated vegetable fat. The mice were housed two to a cage and "auditory" stimuli or electric shock were applied at regular intervals from the fifteenth to the thirtieth week of the experiment. The incidence of spontaneous convulsions and convulsive deaths was

reduced in mice fed restricted amounts of the basal diet. Hydrogenated vegetable fat accelerated the onset and increased the incidence of convulsive deaths as compared to results obtained with lard. M. SILBERBERG

Parkinsonian manifestations have been one of the complications associated with the use of chlorpromazine in the treatment of neuropsychiatric cases. The rise in plasma copper associated with the drug differs from Wilson's disease in which there is a decrease in total plasma copper concentration with an increase in direct-reading copper. However, involvement of basal ganglia by copper deposition may account for the manifestations described below.

Alteration of Copper Metabolism in Chlorpromazine-Treated Cases. H. Azima and. A. Richman. *A. M.A. Arch. Neurol. & Psychiat.* 75: 163, 1956.

The authors have demonstrated that some cases treated with chlorpromazine show alterations of copper metabolism, and a syndrome similar in some respects to Wilson's disease (hepatolenticular degeneration). The combination of extrapyramidal signs and liver damage in chlorpromazine-treated cases led to this study of copper metabolism.

Plasma copper of 25 nonselected psychiatric patients (average age, 40) who received chlorpromazine (from 75 to 400 mg per day) was measured before the treatment and at the end of the first, third, and fifth weeks after the beginning of the treatment. Liver function tests were performed once a week. Plasma copper was measured by the sodium diethyldithiocarbamate method. A control group consisted of 25 psychiatric cases which received other forms of treatment.

The majority of cases which were treated with chlorpromazine showed a gradual rise in plasma copper; in 12 cases the rise was definitely abnormal (beyond 160/100 cc). Of these 12 cases, five manifested extrapyramidal signs, consisting of cogwheel rigidity, tremor, loss of associated movements, mask-like facies, etc. In one case which evidenced liver damage there was a moderate rise in plasma copper. In no case was there a combination of plasma copper rise, liver damage, and extrapyramidal signs. No case manifested neurologic signs without a rise in plasma copper. There was no correlation between the dosage of chlorpromazine and the appearance or intensity of extrapyramidal complications. In the control group there was no persistent pattern, no rise in plasma copper, or any neurologic changes. Extrapyramidal signs disappeared within a month after the cessation of chlorpromazine therapy in all cases.

Chlorpromazine provokes an alteration of copper metabolism in the body. Because the indirect-reacting fraction of plasma copper was not measured in the present series, it cannot be definitely concluded that there is an identity between the changes produced by chlorpromazine and by Wilson's disease. However, the similarity of the two conditions cannot be excluded because of the clinical picture of chlorpromazine complications (hepatic and lenticular), which is similar to that in Wilson's disease, and the fact that the copper metabolism was abnormal only in those cases which showed neurologic signs. S. W. CONRAD

The poor nutritional status of patients with multiple sclerosis produces conditions which favor impairment of carbohydrate tolerance. There is apparently no metabolic disturbance associated with the disease itself which is capable of producing this alteration.

Carbohydrate Metabolism and Nutritional State in Multiple Sclerosis. H. Droller and I. J. N. Powell. *J. Chronic Dis.* 4: 283, 1956.

Disturbances in carbohydrate metabolism caused by thiamine deficiency have been incriminated as a cause of multiple sclerosis. The validity of this theory was investigated in 10 male and female patients ranging in age from 35 to 61 years, ill with the disease for periods of 6 to 21 years. The general nutritional levels of all these patients was poor; their diets were particularly low in fat and protein, also, in the case of women, in carbohydrate. Glucose tolerance tests which were abnormal and resembled those seen in starvation, could be rectified by glucose feeding. There was no evidence of thiamine deficiency. Satisfactory basic nutritional conditions have to be established before the significance of tests indicating disturbances in carbohydrate metabolism in such patients can be evaluated.

M. SILBERBERG

Myopathies have been described in a wide variety of animals maintained on vitamin E-deficient diets. These conditions are corrected by the administration of α-tocopherols. Unfortunately, this therapy has not proved successful in clinical experience.

Comparative Effects of Alpha-Tocopherol, DPPD and Other Antioxidants on Muscular Dystrophy in Guinea Pig. R. Shull, R. B. Alfin-Slatter, H. J. Deuel, Jr., and B. H. Ershoff. *Proc. Soc. Exp. Biol. Med.* 95: 263, 1957.

The effects of 0.025 per cent of the antioxidants N,N'-diphenyl-*p*-phenylenediamine, 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinolone and 2,5-di-tert-butylhydroquinone were tested in guinea pigs kept on a strictly vitamin E-free diet. These supplements were added to the diet which was given on alternate dates, leftovers being removed in order to minimize oxidative changes in the diet. The experiments were carried through one year or until death. For 12 weeks, the animals receiving the above antioxidants showed no difference from controls receiving a vitamin E-free diet or diets containing supplements of α-tocopherol. From the thirteenth week on, animals kept on the unsupplemented vitamin E-free diet developed deficiency symptoms; those receiving antioxidants did not develop muscular dystrophy before the twenty-fifth week, while a supplement of α-tocopherol provided full protection against the development of muscular dystrophy. M. SILBERBERG

Neurologic syndromes associated with chronic alcoholism have been attributed to secondary vitamin deficiency states and to the direct toxic effect of high ethanol concentrations upon the tissues. Wernicke's syndrome may be improved by administration of large doses of niacinamide and thiamine.

The Changing Neuropathologic Picture of Chronic Alcoholism. K. T. Neubuerger. *A. M. A. Arch. Path.* 63: 1, 1957.

The brains of 42 alcoholics ranging from 30 to 70 years of age, one-third of them women, were studied microscopically. Polioencephalitis hemorrhagica superior (Wernicke's disease) has become very rare during recent years. However, a cerebellar lesion was rather common consisting of a more or less severe nonspecific degeneration of the granular cell layer.

M. SILBERBERG

Circulatory Studies in Wernicke's Encephalopathy with Special Reference to the Occurrence of a State of High Cardiac Output and Postural Hypotension. M. A. Gravallese, Jr., and M. Victor. *Circulation* 15: 836, 1957.

The circulation was studied in 13 chronic alcoholic patients with acute Wernicke's encephalopathy, attributed to thiamine deficiency. In four out of seven patients the resting cardiac output was elevated out of proportion to oxygen consumption and associated with low peripheral vascular resistance, sinus tachycardia, and increased stroke volume. The abnormalities reverted to normal after treatment with thiamine. In two patients cardiac output was low and unaffected by treatment. Heart failure was not encountered.

The cardiovascular response to tilting was studied in 12 patients; postural hypotension was found in seven. This may be responsible for the dizziness and syncope which occur in this disease.

The authors suggest that the circulatory abnormalities reflect a state of peripheral vasodilation specifically related to thiamine deficiency. W. H. ABELMANN

It is difficult to accept the view presented in the first of the two following papers that vitamins have little to do with peripheral nerve lesions. The fact that vitamin administration does not correct neuropathy is inadequate evidence upon which to base such an opinion. In the second paper neuropathologic changes are observed; whether these are due to vitamin deficiency or inanition cannot be determined.

Malnutrition and Peripheral Nerve Disorders. H. Luckner, and R. Magun. *German Med. Monthly.* 2:97, 1957.

The authors reinvestigated the problem of nutritional peripheral neuropathy and came to the conclusion that there is no reason to assume a causal relationship between malnutrition and the nerve lesions. A survey was made of 191 cases seen between 1943 and 1955. During the years in which there was an increase of polyneuropathy there was also an increased incidence of diphtheria. In 25 cases of diabetic neuropathy, there was neither an insufficient caloric intake nor vitamin deficiency which could be implicated. It should be pointed out, however, that the authors put a great deal of stock in the Rohrer Index which is a formula relating body weight to body length, and is used by them as an indication of "malnutrition."

The authors also carried out a number of experiments on rats with the object of producing experimental polyneuropathy but were unable to do so. No form of thiamine deficiency led to neuropathy. After producing the edematous and cardiovascular forms of beriberi by a diet deficient in protein and thiamine, it was not possible to produce peripheral nerve lesions by withholding several other dietary factors at one time.

It is said that the basis of the treatment of polyneuropathy and other nervous disorders with vitamins was the production in animals by means of thiamine deficiency of a clinical picture which was interpreted as "polyneuritis" and which could be cured by vitamin B_1. This interpretation is said to be false. The authors conclude: "It is a shattering experience to discover that the routine of administering vitamins for peripheral nerve lesions, observed in many places and over many years, has, in fact, never shown any reproducible success." The authors make the flat statement that vitamins influence neither the course nor the duration of peripheral nerve disorders.

This is a provocative paper, but one must have some reservations because it seems that the authors' conclusions are stated as positively as the conclusions of others which the paper itself criticizes. S. O. WAIFE

Nutritional Neuropathy. J. G. K. North, and H. M. Sinclair. *A. M. A. Arch. Pathol.* 62: 341, 1956.

Seventy-five adult albino rats six months of age were made thiamine-deficient and divided into three groups varying in the degree of thiamine deficiency. The animals, weight controls and pair-fed controls were kept alive for periods up to 156 days depending upon the degree of deficiency. The nervous system of the rat was found to be fairly resistant to chronic thiamine deficiency, but if the latter was of sufficient degree and duration, degeneration of myelin and axis cylinders was noted in the sciatic and posterior tibial nerves. However, since inanition may produce slight regressive changes in the fibers, the question may be raised as to whether or not the changes are specifically due to thiamine deficiency. M. SILBERBERG

That a center for control of appetite exists in the mediolateral hypothalmic areas seems to be established by extensive animal experimentation. The evidence for this concept and its important implications are reviewed in the following paper.

Neural Basis of Hunger, Appetite and Satiety. J. R. Brobeck. *Gastroenterology* 32: 169, 1957.

The quantitative regulation of food intake appears to be predominantly the function of the hypothalamus. The fact that sensations from the gastrointestinal tract have little effect on feeding is demonstrated by the relatively normal caloric intake observed in animals whose gastrointestinal tracts have been completely denervated. It is significant that bilateral injury to the tuberal portion of the hypothalamus near the pituitary stalk and close to the midline consistently causes an increase in food intake with resulting obesity often persisting throughout the life of the animal. In contrast, injury to the lateral hypothalamic area causes a cessation of all feeding despite otherwise normal behavior, with resulting starvation, the life of the animal being preserved only by tube feeding. Therefore, the medial hypothalamic area appears to be a "satiety" center and the lateral hypothalamic region, a "feeding" center. When amphetamine is given an animal the electroencephalogram will record an alteration in the record from the medial region of the hypothalamus suggesting an increase in the activity of the satiety center as an explanation of the anorexogenic effects of the drug. There is suggestive evidence that reduction in feeding activity occurs when the satiety center is stimulated by signals arising from the chewing and swallowing of food, by filling of the stomach, by increasing availability of glucose to the nervous system, by increasing thirst, and by rising body temperature.

The author has summarized the available information on the neural controls of hunger and appetite in a manner which should be especially useful to anyone interested in research in obesity and anorexia.

J. B. HAMMOND